PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. PCT/US 93/00030

International Filing Date 12 JAN 1993

PCT INTERNATIONAL APPLICATION RO/US

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 92,307-A; D02 CIP PCT

DELETED BY RO/US

Box No. I TITLE OF INVENTION

TREATMENT FOR ASTHMA

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BIOGEN, INC.
14 Cambridge Center
Cambridge, Massachusetts 02142
United States of America

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LOBB, Roy R.
62 Loring Street
Westwood, Massachusetts 02090
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is **applicant** for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

PCT/US 93/00030

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: [X] agent [ ] common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

McNICHOLAS, Janet M.
ALLEGRETTI & WITCOFF, LTD.
Ten South Wacker Drive
Chicago, Illinois 60606
UNITED STATES OF AMERICA

Telephone No.: 312-715-1000

Fascimile No.: 312-715-1234

Teleprinter No.:

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

[X] **EP European Patent**: **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FR** France, **GB** United Kingdom, **GR** Greece, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

[ ] **OA OAPI Patent:** Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Congo, Côte d'Ivoire, Gabon, Guinea, Mali, Mauritania, Senegal, Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

[ ] **AT** Austria .....
[X] **AU** Australia .....
[ ] **BB** Barbados
[ ] **BG** Bulgaria .....
[ ] **BR** Brazil .....
[X] **CA** Canada
[ ] **CH and LI** Switzerland and Liechtenstein
[ ] **CS** Czechoslovakia
[ ] **DE** Germany .....
[ ] **DK** Denmark .....
[ ] **ES** Spain .....
[ ] **FI** Finland
[ ] **GB** United Kingdom
[ ] **HU** Hungary .....
[X] **JP** Japan .....
[ ] **KP** Democratic People's Republic of Korea
.....
[ ] **KR** Republic of Korea .....
[ ] **LK** Sri Lanka
[ ] **LU** Luxembourg .....
[ ] **MG** Madagascar
[ ] **MN** Mongolia .....
[ ] **MW** Malawi .....
[ ] **NL** Netherlands
[ ] **NO** Norway
[ ] **PL** Poland .....
[ ] **RO** Romania
[ ] **RU** Russian Federation
[ ] **SD** Sudan
[ ] **SE** Sweden
[X] **US** United States of America .....
(Continuation-in-part)
.....

RO/45

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

[X] ....NEW ZEALAND ....NZ▲.....
[ ] .....
[ ] .....
[ ] .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box ***If the Supplemental Box is not used, this sheet need not be included in the request.***

Use this box in the following cases:

1. If, in any of the Boxes, the space is insufficient to furnish all the information:

in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;

in particular:

(i) if more than three persons are involved as applicants and/or inventors and no "continuation sheet" is available:

in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:

in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State or States (and/or, where applicable, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:

in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State or States (and/or, where applicable, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents:

in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," "certificate of addition," or "inventor's certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":

in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if there are more than three earlier applications whose priority is claimed:

in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.

2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:

in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.

CONTINUATION OF BOX NO. V

United States of America, Application Serial No. 821,768, filed 13 January 1992 (13.01.92)

PCT/US 93/00031

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
(1) US	13 January 1992 (13.01.92)	821,768	
(2)			
(3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to transmit to the International Bureau a certified copy of the earlier application(s) identified above at item(s): (1)

Box No. VII EARLIER SEARCH

Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:

Country (or regional Office):	Date (day/month/year): (13.01.92)	Number:
United States of America	13 January 1992	821,768

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	: 4	sheets
2. description	: 30	sheets
3. claims	: 3	sheets
4. abstract	: 1	sheets
5. drawings	: 6	sheets
Total	: 44	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) (specify):
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing
8. ☒ other (specify): PCT International Application Transmittal Letter

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Janet M. McNicholas
Janet M. McNicholas
[Reg. No. 32,918]

For receiving Office use only

1. Date of actual receipt of the purported international application: 39 Rec'd PCT/PTO 12 JAN '93
2. Drawings ☒ received ☐ not received
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA/EP ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

DELETED BY RO/US

PCT/US 93/00030
RO/US 26 FEB 1993

PCT Application of:
BIOGEN, INC.

Title: **Treatment for Asthma**

Serial No.: **PCT/US93/00030**
Filing Date: **January 12, 1993**
Case No.: **92,307-A**

I, **Leon R. Yankwich** am the **Patent Counsel** of **BIOGEN, INC.**, a corporation of the State of Massachusetts located at 14 Cambridge Center, Cambridge, Massachusetts 02142, the owner and assignee of all right, title and interest in the above-identified application, and am authorized to grant this power of attorney.

I, **Roy R. Lobb**, am the inventor of the above-entitled invention, residing at 62 Loring Street, Westwood, Massachusetts 02090, United States of America.

The undersigned hereby appoints the following:

John J. McDonnell	Reg. No. 26,949
Janet M. McNicholas	Reg. No. 32,918
Wayne A. Keown	Reg. No. 33,923
John P. Iwanicki	Reg. No. 34,628
Steven J. Sarussi	Reg. No. 32,784
Kevin E. Noonan	Reg. No. 35,303
Dale A. Malone	Reg. No. 32,155
Barbara A. Heaphy	Reg. No. 34,619

the mailing address and telephone number of each of whom is ALLEGRETTI & WITCOFF, LTD., 10 South Wacker Drive, Chicago, Illinois 60606, United States of America, and (312) 715-1000, with full power of substitution and revocation to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith, and throughout the world with respect thereto for all corresponding applications.

ALLEGRETTI & WITCOFF, LTD.
10 South Wacker Drive
Chicago, Illinois 60606
(312) 715-1000

PCT/US 93/00030
RO/US 26 FEB 1993

Applicant: **BIOGEN, INC.**

By: _____
Leon R. Yankwich
Patent Counsel

Post Office Address: **14 Cambridge Center**
Cambridge, Massachusetts 02142
United States of America

Date: 1/28/93

_____
Roy R. Lobb
Inventor

Post Office Address: **62 Loring Street**
Westwood, Massachusetts 02090
United States of America

Date: 1/27/93

ALLEGRETTI & WITCOFF, LTD.
10 South Wacker Drive
Chicago, Illinois 60606
(312) 715-1000

PCT

DEMAND

CHAPTER II

Demand under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 92,307-A; (D002 CIP PCT)

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/US93/00030	12 January 1993	12 January 1992

Title of invention

TREATMENT FOR ASTHMA

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BIOGEN, INC.
14 Cambridge Center
Cambridge, Massachusetts 02142
UNITED STATES OF AMERICA

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LOBB, Roy R.
62 Loring Street
Westwood, Massachusetts 02090
UNITED STATES OF AMERICA

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

☐ Further applicants are indicated on a continuation sheet.

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, i addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

McDONNELL, John J.
McNICHOLAS, Janet J.
ALLEGRETTI & WITCOFF, LTD.
Ten South Wacker Drive
Chicago, Illinois 60606
UNITED STATES OF AMERICA

Telephone No.: 312-715-1000

Facsimile No.: 312-715-1234

Teleprinter No.:

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is us instead to indicate a special address to which correspondence should be sent.

Box No. IV STATEMENT CONCERNING AMENDMENTS

The applicant wishes the International Preliminary Examining Authority*

(i) [X] to start the international preliminary examination on the basis of the international application as originally file

(ii) [ ] to take into account the amendments under Article 34 of

- [ ] the description (amendments attached)
- [ ] the claims (amendments attached)
- [ ] the drawings (amendments attached)

(iii) [ ] to take into account any amendments of the claims under Article 19 filed with the International Bureau (a copy attached).

(iv) [ ] to disregard any amendments of the claims made under Article 19 and to consider them as reversed.

(v) [ ] to postpone the start of the international preliminary examination until the expiration of 20 months from the prior date unless that Authority receives a copy of any amendments made under Article 19 or a notice from the applic that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the ti limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international applicati as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the internatior application under Article 34 are received by the International Preliminary Examining Authority before it has begun to dr up a written opinion or the international preliminary examination report, as so amended.

Box No. V ELECTION OF STATES

The following designated States are hereby elected:

(i) [ ] all eligible States *(i.e., all designated States bound by Chapter II of the PCT).*

(ii) [X] the States indicated in the Supplemental Box No. V.

Supplemental Box No. ELECTION OF STATES

This Supplemental Box is to be used only if the check-box "(ii)" in Box No. V is marked.
If this Supplemental Box is not used, do not include this sheet in the demand.

The following designated States are hereby elected :

Regional Patent

[X] **EP European Patent:** AT Austria. BE Belgium. DE Germany. DK Denmark. FR France. GB United Kingdom. IT Italy. LU Luxembourg. MC Monaco. NL Netherlands. SE Sweden.
and any other State which is a Contracting State of the European Patent Convention and of the PCT (including Chapter II thereof)

[ ] **OA OAPI Patent:** Benin. Burkina Faso. Cameroon. Central African Republic. Chad. Congo. Côte d'Ivoire. Gabon. Guinea. Mali. Mauritania. Senegal. Togo.
and any other State which is a member State of OAPI and a Contracting State of the PCT (including Chapter II thereof)

National Patent

- [ ] **AT** Austria
- [X] **AU** Australia
- [ ] **BB** Barbados
- [ ] **BG** Bulgaria
- [ ] **BR** Brazil
- [X] **CA** Canada
- [ ] **CS** Czechoslovakia
- [ ] **DE** Germany
- [ ] **DK** Denmark
- [ ] **FI** Finland
- [ ] **GB** United Kingdom
- [ ] **HU** Hungary
- [X] **JP** Japan
- [ ] **KP** Democratic People's Republic of Korea
- [ ] **KR** Republic of Korea
- [ ] **LK** Sri Lanka
- [ ] **LU** Luxembourg
- [ ] **MG** Madagascar
- [ ] **MN** Mongolia
- [ ] **MW** Malawi
- [ ] **NL** Netherlands
- [ ] **NO** Norway
- [ ] **PL** Poland
- [ ] **RO** Romania
- [ ] **RU** Russian Federation
- [ ] **SD** Sudan
- [ ] **SE** Sweden
- [X] **US** United States of America

(Continuation-in-Part)

Check-boxes reserved for electing States (for the purposes of a national patent) which have become party to the PCT (including Chapter II thereof) or bound by Chapter II of the PCT after issuance of this sheet:

- [X] NEW ZEALAND .....
- [ ] .....
- [ ] .....
- [ ] .....

See Notes to the dema

Box No. VI CHECKLIST

The demand is accompanied by the following documents for the purposes of international preliminary examination:

			For International Preliminary Examining Authority use only: received	not received
1. amendments under Article 34				
description	:	sheets	☐	☐
claims	:	sheets	☐	☐
drawings	:	sheets	☐	☐
2. letter accompanying amendments under Article 34	:	sheets	☐	☐
3. copy of amendments under Article 19	:	sheets	☐	☐
4. copy of statement under Article 19	:	sheets	☐	☐
5. other *(specify)*:	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☒ fee calculation sheet
5. ☒ other *(specify)*: TRANSMITTAL LETTER

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

John J. McDonnell
U.S. Reg. No. 26,949

Janet M. McNicholas
U.S. Reg. No. 32,918

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date. ☐ The applicant has been informed accordingly.

For International Bureau use only

Demand received from IPEA on:

IN THE PATENT COOPERATION TREATY
EUROPEAN PATENT OFFICE
(Case No. 92,307-A; D002 CIP PCT)

Applicant:	Biogen, Inc.
International Application No.:	PCT/US93/00030
International Filing Date:	12 January 1993
Priority Date Claimed:	13 January 1992
Primary Examiner:	M. Herrero

REPLY TO WRITTEN OPINION

BY FACSIMILE (+49-89) 2399-4465

IPEA/European Patent Office
ATT: M. Herrero, Examiner
D-80298 Munich, Germany

Sir:

This Reply is in response to the Written Opinion mailed from the International Preliminary Examining Authority (IPEA) on 13 October 1993 for the above-identified application. A confirmation copy of this Reply will follow shortly.

IN THE CLAIMS

Please renumber original claim 20 as new claim 21 and insert the following new claim 20 into the present application. A substitute page 33A, containing the amendments, is attached.

-- 20. A method for the treatment of asthma comprising administering to a mammal suffering from asthma a composition comprising anti-VLA-4 antibody HP1/2 or a fragment thereof capable of binding to VLA-4. --

IN THE DESCRIPTION

Please amend the description as shown below and replace page 6 with replacement page 6A.

Page 6, line 13, after "Figure 4" insert --(comprised of Figures 4A, 4B, 4C and 4D)--;

to page 14, line 5, and references cited therein. Accordingly, the Applicant submits that because the aforementioned materials are commonly involved in the interference of VLA-4-mediated binding and thus would have utility in asthma treatment, unity of invention exists among the Applicants' claimed embodiments.

CONCLUSIONS

Applicants respectfully submit that the present claims are drawn to novel and inventive subject matter having industrial applicability, and that the present amendments render the claims and description compliant with PCT article 33(3). Withdrawal of the objections and issuance of a statement to this effect are respectfully requested.

Respectfully submitted,
ALLEGRETTI & WITCOFF, Ltd.
10 S. Wacker Drive
Chicago, Illinois 60606 USA

Dated: January 6, 1994

By: _____
John J. McDonnell
Reg. No. 26,949

F:\ATTY\EXM\BIOGEN\92307\PTO\92307B.RES

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Mrs J.M. McNicholas
ALLEGRETTI & WITCOFF, LTD.
10 South Wacker Drive
CHICAGO, ILLINOIS 60606
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF RECEIPT OF DEMAND

(PCT Rule 61.1(b), first sentence
and Administrative Instructions, Section 601)

Date of mailing *(day/month/year)*: 27.08.93

Applicant's or agent's file reference: [redacted]

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 93/00030	12/01/1993	13/01/1992

Applicant

BIOGEN INC. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

06/08/1993

2. This date of receipt is:

☒ the actual date of receipt of the demand.

☐ the date on which the proper corrections to the demand were timely received.

3. ☐ This date is **AFTER** the expiration of 19 months from the priority date.

Attention: The election(s) made in the demand does (do) not have the effect of postponing the commencement of the national phase until 30 months from the priority date (or later in some Offices)(Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22).

For details, see Annex B to Form PCT/IB/301 sent by the International Bureau and Volume II of the PCT Applicant's Guide.

☐ This notification confirms the information given in person or by telephone on:

IE IRELAND + PT PORTUGAL have been inserted as EP-Patent.
The priority date has been corrected ex officio (13.01.92)

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/

European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer

H.-P. Dietenhofer

Form PCT/IPEA/402 (July 1992) P20452 (10/08/1993)

page 6, line 14, after "total cells" insert --(Fig. 4A)--;

page 6, line 15, after "lymphocytes" insert --(Fig. 4B)--; after "neutrophils" insert --(Fig. 4C)--; and after "eosinophile" insert --(Fig. 4D)--.

REMARKS

Claims 1-20 were pending in this application. Old claim 20 was renumbered as claim 21 and a new claim 20 was inserted into the application. Support for new claim 20 can be found in claims 1 and 5. Furthermore, the description was corrected to clarify references to the four graphs filed as formal drawings Figures 4A, 4B, 4C, and 4D (filed as originally as Figure 4).

Substitute sheets 6A and 33A which correspond to original pages 6 and 33, respectively, and reflect the aforementioned amendments are attached. No new subject matter has been added as a result of the amendments.

1. Applicant's claimed subject matter satisfies the inventive step requirement of PCT Article 33(3).

Claims 1-20 stand objected to as lacking an inventive step as required under Article 33(3) PCT. In making the objection, the Examiner relied on the disclosures of D1 (Weller et al. (1991) Proc. Natl. Acad. Sci. USA 88: 7430-7433) and D2 (Wegner et al. (1990) Science 247: 456-459). The D1 reference relates to VCAM-1/VLA-4 pathway while the D2 reference relates to ICAM-1/LFA-1. The Examiner contends that one of ordinary skill would have been motivated to apply D1 teachings with respect to anti-VLA-4 blocking of VCAM-1 mediated adhesion to D2 teachings with respect to anti-ICAM-1 treatment of asthma and thus arrive with Applicants' invention. Applicants respectfully traverse this objection.

The ICAM-1/LFA-1 adhesion pathway and the VCAM-1/VLA-4 adhesion pathway are two completely different, complex biological systems. There is nothing in D1 or D2 teachings that would suggest or imply that the two adhesion pathways are interchangeable in vivo or that discoveries relating to ICAM-1/LFA-1 interactions could be applicable to or predictive of the molecular interactions between VCAM1 and VLA-4. Indeed, blocking VLA-4 adhesion of circulating leukocytes would not be expected

to inhibit, for example, neutrophil recruitment (since neutrophils do not express VLA-4) and would not be expected to inhibit eosinophil recruitment (since eosinophils could be recruited via ELAM-1 and ICAM-1 in addition to VCAM-1). *See for example* page 7430, left column, in the D1 reference.

Additionally, while the D1 reference hints that the VCAM1/VLA-4 pathway might be involved in asthma, it does not suggest that blocking the pathway would be an effective treatment for asthma. Because it was known that alternative adhesion pathways exist *in vivo*, one could not have hoped to treat asthma by blocking leukocyte recruitment alone, using one antibody, particularly an anti-VLA-4 antibody. Accordingly, a person of ordinary skill in the art would not apply teachings relating to the activity of anti-VLA-4 antibodies to methods involving anti-ICAM-1 or anti-CD18 antibodies. Withdrawal of the objection is in order and is respectfully urged.

2. Response concerning certain observations of the international application

The Examiner contends that the Applicants' invention relates specifically to the use of antibodies recognizing VLA-4 in the treatment of asthma and thus objected to certain embodiments, e.g., polypeptides and molecules capable of binding to the alpha subunit of VLA-4, recited in claims 12, 13, 17 and 18 as being non-unitary with the rest of the main subject matter of the claims. Applicants respectfully traverse the objection.

The Applicants' invention, as presently claimed, relates to a method for treating asthma which employs substances which interfere with VLA-4-mediated binding. In addition to anti-VLA antibodies, the Applicants contemplate that polypeptides and molecules which are capable of inhibiting or blocking VLA-4-mediated binding would also be useful in the treatment of asthma. For example, the description teaches that certain polypeptides (e.g., soluble VCAM-1 or fragments thereof which compete for the VLA-4 binding site) and molecules (e.g., oligosaccharides which mimic the binding domain of a VLA-4 ligand) are expected to have a therapeutic effect against asthma similar to that of anti-VLA-4 antibodies. See the Applicants' description on page 13, line 20

from 0.05 to 5.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

18. The method of Claim 17, wherein the composition is administered at a dosage so as to provide 1.0-2.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

19. The method according to Claim 12, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the mammal of at least 10 μg/ml over a period of 7 days.

20. A method for the treatment of asthma comprising administering to a mammal suffering from asthma a composition comprising anti-VLA-4 antibody HP1/2 or a fragment thereof capable of binding to VLA-4.

21. A pharmaceutical composition effective to attenuate late phase response or significantly reduce airway hypersensitivity in an asthmatic mammal consisting essentially of a monoclonal antibody recognizing VLA-4 in a pharmaceutically acceptable carrier.

Figure 2 is a graph depicting plasma concentration of monoclonal antibody HP1/2 (intravenous) in sheep, measured over time after initial administration.

Figure 3 is a graph depicting the effect of monoclonal antibody HP1/2 (intravenous) on airway hyperresponsiveness in dual responder sheep. Airway responsiveness, measured in breath units (BU) of cumulative breaths of a 1% weight/volume carbachol solution (a known bronchoconstrictor) that increases specific lung resistance 400% over the value obtained using diluent alone. Asterisks indicate statistically significant results.

Figure 4 (comprised of Figures 4A, 4B, 4C and 4D) is a series of four graphs showing the total cells (Fig. 4A) and the levels of different leukocytes (lymphocytes (Fig. 4B), neutrophils (Fig. 4C), and eosinophils (Fig. 4D)) detected by bronchoalveolar lavage in allergic sheep challenged with Ascaris suum antigen alone and after pretreatment with monoclonal antibody HP1/2 (intravenous). Total cells, and the percentage of total cells that were lymphocytes or neutrophils or eosinophils, were measured at 4-hour, 8-hour, 24-hour, 48-hour and 1-week time points post allergen challenge.

Figure 5 is a graph depicting the effect of monoclonal antibody HP1/2 (16 mg, aerosol) and 1E6 (16 mg, aerosol) on the response to allergen (Ascaris suum antigen) in dual responder allergic sheep. Percentage change in specific lung resistance (SR_L) is measured over time post allergen challenge. Asterisks indicate statistically significant results.

Figure 6 is a graph depicting the effect of monoclonal antibody HP1/2 (16 mg, aerosol) and 1E6 (16 mg aerosol) on airway hyperresponsiveness in dual responder

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
Washington, D.C.

in its capacity as elected Office

Date of mailing: 01 September 1993 (01.09.93)	
International application No.: PCT/US93/00030	Applicant's or agent's file reference: 92,307-A; DO
International filing date: 12 January 1993 (12.01.93)	Priority date: 13 January 1992 (13.01.92)
Applicant: LOBB, Roy, R.	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   06 August 1993 (06.08.93)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

A. Grocq

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
Washington, D.C.

in its capacity as elected Office

Date of mailing: 27 August 1993 (27.08.93)	
International application No.: PCT/US93/00330	Applicant's or agent's file reference: 16071
International filing date: 13 January 1993 (13.01.93)	Priority date: 14 January 1992 (14.01.92)
Applicant: ZACKS, Shelemyahu et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 July 1993 (27.07.93)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

B. Morariu

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

United States Patent and Trademark Office
Washington, D.C.

in its capacity as elected Office

NOTIFICATION CONCERNING DOCUMENT TRANSMITTED

Date of mailing: 18 April 1994 (18.04.94)	
International application No.: PCT/US93/00030	International filing date: 12 January 1993 (12.01.93)
Applicant: BIOGEN, INC. et al	

The International Bureau transmits herewith the following documents and number thereof:

_____ copy of the international preliminary examination report and annexes (Article 36(3)(a))

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorised officer:

C. Carrié

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

PCT

RECD 15 APR 1994
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 92,307-A	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US 93/00030	International filing date (day/month/year) 12/01/1993	Priority date (day/month/year) 13/01/1992
International Patent Classification (IPC) or national classification and IPC A61K39/395		
Applicant BIOGEN INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings amended during international preliminary examination and/or containing rectifications made before this Authority.

These annexes consists of a total of 7 sheets.

3. This report contains indications and corresponding pages relating to the following items:

- I [X] Basis of the report
- II [ ] Priority
- III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [X] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/08/1993	13.04.94
Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer M. Herrero

Intern. application No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT PCT/US93/00030

I. Basis of the report

1. This report has been drawn up on the basis of:

[ ] the international application as originally filed.

[x] the description, pages 1-5, 7-25_____, as originally filed,
pages _____, filed with the demand,
pages 26-30_____, filed with the letter of 21.04.93,
pages 6A_____, filed with the letter of 11.01.94,

[x] the claims, No. 1-17(part)_____, as originally filed,
No. _____, as amended under Article 19,
No. _____, filed with the demand,
No. 17(part)-21_____, filed with the letter of 11.01.94,
No. _____, filed with the letter of _____,

[x] the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig 1/6-6/6 (RO/US)_____, filed with the letter of 26.02.93,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of: pages: _____
sheets of drawings/figures No.: _____.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed:

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

III. Non-establishment of opinion with regard to ~~novelty, inventive step and~~ industrial applicability

The question whether the claimed invention appears ~~to be novel, to involve an inventive step (to be non-obvious), or~~ to be industrially applicable ~~have~~ has not been and will not be examined in respect of:

[ ] the entire international application,

[x] claims Nos. 1-20

because:

[x] the said international application, or the said claims Nos. 1-20 relate to the following subject matter which does not require an international preliminary examination (specify):

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods (PCT Rule 67.1(iv)). See also Section V, Citations and Explanations.

[ ] the description, claims or drawings (indicate particular elements below) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (specify):

[ ] the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

[ ] no international search report has been established for said claims Nos. _____.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-21	YES
	Claims	NO
Inventive Step (IS)	Claims 1-21	YES
	Claims	NO
Industrial Applicability (IA)	Claims 21	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

1. The subject-matter at present claimed meets the criteria set forth in Article 33(2)-33(3) PCT since the available prior art does not teach or suggest the claimed invention.

The application discloses a method for treating asthma in a mammal which method relies on the blockage of the VCAM1/VLA-4 adhesion pathway of circulating leukocytes and employs substances which specifically interfere with VLA-4-mediated binding. Molecules contemplated as capable of inhibiting or blocking said VLA-4 mediated binding are in particular anti-VLA-4 antibodies and VLA-4 binding fragments thereof capable of binding to the α_4 subunit of VLA-4, and certain polypeptides, e.g. soluble VCAM-1 or fragments thereof which compete for the VLA-4 binding site, and oligosaccharides which mimic the binding domain of a VLA-4 ligand.

2. For the assessment of the present Claims 1-20 on the question whether they are industrially applicable, no

unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. It is clearly pointed out in the description (see for instance page 12, lines 15-17) that for the purposes of the present invention, antibodies capable of binding to the α_4 subunit of VLA-4 must be employed. Since independent Claim 1 does not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6(3)(b) PCT that any independent claim must contain all the technical features essential to the invention.

2. The terms "a polypeptide" and "a small molecule" as presently used in the independent Claim 12 are vague and indefinite and, as such, render the scope of the claim unclear; accordingly, the claim does not satisfy the requirement of Article 6 PCT. In the light of the description it could be suggested to clarify said vague terms using the corresponding expressions "a polypeptide which competes for the VLA-4 binding site" (page 13, line 26) and "a small molecule that mimic the binding domain of a VLA-4 ligand" (page 13, lines 29-30).

Figure 2 is a graph depicting plasma concentration of monoclonal antibody HP1/2 (intravenous) in sheep, measured over time after initial administration.

Figure 3 is a graph depicting the effect of monoclonal antibody HP1/2 (intravenous) on airway hyperresponsiveness in dual responder sheep. Airway responsiveness, measured in breath units (BU) of cumulative breaths of a 1% weight/volume carbachol solution (a known bronchoconstrictor) that increases specific lung resistance 400% over the value obtained using diluent alone. Asterisks indicate statistically significant results.

Figure 4 (comprised of Figures 4A, 4B, 4C and 4D) is a series of four graphs showing the total cells (Fig. 4A) and the levels of different leukocytes (lymphocytes (Fig. 4B), neutrophils (Fig. 4C), and eosinophils (Fig. 4D)) detected by bronchoalveolar lavage in allergic sheep challenged with Ascaris suum antigen alone and after pretreatment with monoclonal antibody HP1/2 (intravenous). Total cells, and the percentage of total cells that were lymphocytes or neutrophils or eosinophils, were measured at 4-hour, 8-hour, 24-hour, 48-hour and 1-week time points post allergen challenge.

Figure 5 is a graph depicting the effect of monoclonal antibody HP1/2 (16 mg, aerosol) and 1E6 (16 mg, aerosol) on the response to allergen (Ascaris suum antigen) in dual responder allergic sheep. Percentage change in specific lung resistance (SR_L) is measured over time post allergen challenge. Asterisks indicate statistically significant results.

Figure 6 is a graph depicting the effect of monoclonal antibody HP1/2 (16 mg, aerosol) and 1E6 (16 mg, aerosol) on airway hyperresponsiveness in dual responder

SUBSTITUTE SHEET

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Biogen, Inc.
(B) STREET: 14 Cambridge Center
(C) CITY: Cambridge
(D) STATE: Mass
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 02142

(A) NAME: Roy R. Lobb
(B) STREET: 62 Loring Street
(C) CITY: Westwood
(D) STATE: Mass
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 02090

(ii) TITLE OF INVENTION: Treatment for Asthma

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: PCT/US93/00030

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 821768
(B) FILING DATE: 13-JAN-1992

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG159 insert: HP1/2 heavy chain variable region; amino acid 1 is Glu (E) but Gln (Q) may be substituted"

SUBSTITUTE SHEET

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GTC AAA CTG CAG CAG TCT GGG GCA GAG CTT GTG AAG CCA GGG GCC TCA      48
Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser
 2               6                  11                  16

GTC AAG TTG TCC TGC ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT      96
Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr
            21                  26                  31

ATG CAC TGG GTG AAG CAG AGG CCT GAA CAG GGC CTG GAG TGG ATT GGA     144
Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
        36                  41                  46

AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC CCG AAG TTC CAG     192
Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln
    51                  56                  61

GTC AAG GCC ACT ATT ACA GCG GAC ACG TCC TCC AAC ACA GCC TGG CTG     240
Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
66                  71                  76                  81

CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT GCC GTC TAC TAC TGT GCA     288
Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                86                  91                  96

GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC TTC TGG GGC CAA     336
Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln
            101                 106                 111

GGG ACC ACG GTC ACC GTC TCC TCA                                    360
Gly Thr Thr Val Thr Val Ser Ser
        116                 121
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

SUBSTITUTE SHEET

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser
  2                   6                  11                  16

Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr
              21                  26                  31

Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
          36                  41                  46

Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln
      51                  56                  61

Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
 66                  71                  76                  81

Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                  86                  91                  96

Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln
              101                 106                 111

Gly Thr Thr Val Thr Val Ser Ser
          116                 121
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 318 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..318
(D) OTHER INFORMATION: /product= "HP1/2 light chain variable region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG172 insert: HP1/2 light chain variable region"

SUBSTITUTE SHEET

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTT TCA GCA GGA        48
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
 1               5                  10                  15

GAC AGG GTT ACC ATA ACC TGC AAG GCC AGT CAG AGT GTG ACT AAT GAT        96
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
            20                  25                  30

GTA GCT TGG TAC CAA CAG AAG CCA GGG CAG TCT CCT AAA CTG CTG ATA       144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45

TAT TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT CGC TTC ACT GGC       192
Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60

AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG GCT       240
Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
65                  70                  75                  80

GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCG TAC       288
Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr
                85                  90                  95

ACG TTC GGA GGG GGG ACC AAG CTG GAG ATC                              318
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60
```

SUBSTITUTE SHEET

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
65 70 75 80

Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105

SUBSTITUTE SHEET

from 0.05 to 5.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

18. The method of Claim 17, wherein the composition is administered at a dosage so as to provide 1.0-2.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

19. The method according to Claim 12, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the mammal of at least 10 µg/ml over a period of 7 days.

20. A method for the treatment of asthma comprising administering to a mammal suffering from asthma a composition comprising anti-VLA-4 antibody HP1/2 or a fragment thereof capable of binding to VLA-4.

21. A pharmaceutical composition effective to attenuate late phase response or significantly reduce airway hypersensitivity in an asthmatic mammal consisting essentially of a monoclonal antibody recognizing VLA-4 in a pharmaceutically acceptable carrier.

Best Available Copy

PCT

REQUEST

08/256631

For receiving Office use only

58 Rec'd PCT/PTO 12 JUL 1994

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

Applicant's or agent's file reference (if desired) (12 characters maximum) 92,307-A; D002 CIP

Box No. I TITLE OF INVENTION

TREATMENT FOR ASTHMA

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

BIOGEN, INC.
14 Cambridge Center
Cambridge, Massachusetts 02142
United States of America

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (i.e. country) of nationality: US

State (i.e. country) of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

LOBB, Roy R.
62 Loring Street
Westwood, Massachusetts 02090
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality: US

State (i.e. country) of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Best Available Copy

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: [X] agent [ ] common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

McNICHOLAS, Janet M.
ALLEGRETTI & WITCOFF, LTD.
Ten South Wacker Drive
Chicago, Illinois 60606
UNITED STATES OF AMERICA

Telephone No. 312-715-1000

Facsimile No. 312-715-1234

Teleprinter No.

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

[X] **EP European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FR France, GB United Kingdom, GR Greece, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

[ ] **OA OAPI Patent:** Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Congo, Côte d'Ivoire, Gabon, Guinea, Mali, Mauritania, Senegal, Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [ ] AT Austria .....
- [X] AU Australia .....
- [ ] BB Barbados
- [ ] BG Bulgaria .....
- [ ] BR Brazil .....
- [X] CA Canada
- [ ] CH and LI Switzerland and Liechtenstein
- [ ] CS Czechoslovakia
- [ ] DE Germany .....
- [ ] DK Denmark .....
- [ ] ES Spain .....
- [ ] FI Finland
- [ ] GB United Kingdom
- [ ] HU Hungary .....
- [X] JP Japan .....
- [ ] KP Democratic People's Republic of Korea .....
- [ ] KR Republic of Korea .....
- [ ] LK Sri Lanka
- [ ] LU Luxembourg .....
- [ ] MG Madagascar
- [ ] MN Mongolia .....
- [ ] MW Malawi .....
- [ ] NL Netherlands
- [ ] NO Norway
- [ ] PL Poland .....
- [ ] RO Romania
- [ ] RU Russian Federation
- [ ] SD Sudan
- [ ] SE Sweden
- [X] US United States of America (Continuation-in-part) .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [X] NEW ZEALAND .....
- [ ] .....
- [ ] .....
- [ ] .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Best Available Copy

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

Use this box in the following cases:

1. If, in any of the Boxes, the space is insufficient to furnish all the information:	*in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;*
in particular:	
(i) if more than three persons are involved as applicants and/or inventors and no "continuation sheet" is available:	*in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;*
(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State or States (and/or, where applicable, European or OAPI patent) for the purposes of which the named person is applicant;*
(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State or States (and/or, where applicable, European or OAPI patent) for the purposes of which the named person is inventor;*
(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents:	*in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," "certificate of addition," or "inventor's certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":	*in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*
(vi) if there are more than three earlier applications whose priority is claimed:	*in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:	*in such case, write "Statements Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.*

CONTINUATION OF BOX NO. V

United States of America, Application Serial No. 821,768, filed 13 January 1992 (13.01.92)

Best Available Copy

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
(1) US	13 January 1992 (13.01.92)	821,768	
(2)			
(3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to transmit to the International Bureau a certified copy of the earlier application(s) identified above at item(s): (1)

Box No. VII EARLIER SEARCH

Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:

Country (or regional Office):	Date (day/month/year):	Number:
United States of America	13 January 1992	821,768

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	4	sheets
2. description	30	sheets
3. claims	3	sheets
4. abstract	1	sheets
5. drawings	6	sheets
Total	44	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) (specify):
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing
8. ☒ other (specify): PCT International Application Transmittal Letter

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Janet M. McNicholas

Janet M. McNicholas
Reg. No. 32,918

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received ☐ not received
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA / ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

58 Rec'd PCT/PTO 12 JAN 1994

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 39/395

A1

(11) International Publication Number: WO 93/13798

(43) International Publication Date: 22 July 1993 (22.07.93)

(21) International Application Number: PCT/US93/00030

(22) International Filing Date: 12 January 1993 (12.01.93)

(30) Priority data:
821,768 13 January 1992 (13.01.92) US

(60) Parent Application or Grant
(63) Related by Continuation
US 821,768 (CIP)
Filed on 13 January 1992 (13.01.92)

(71) Applicant *(for all designated States except US)*: BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : LOBB, Roy, R. [US/US]; 62 Loring Street, Westwood, MA 02090 (US).

(74) Agent: McNICHOLAS, Janet, M.; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).

(81) Designated States: AU, CA, JP, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: TREATMENT FOR ASTHMA

(57) Abstract

A method for the treatment of asthma is disclosed. The method comprises administration of an antibody, polypeptide or other molecule recognizing VLA-4, a protein expressed on the surface of certain leukocytes such as eosinophils.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/00030

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A2, 0 330 506 (DANA-FABER CANCER INSTITUTE) 30 August 1989 (30.08.89), claims. --	20
P,X	WO, A1, 92/00 751 (NOVO NORDISK A/S) 23 January 1992 (23.01.92), claims 1,7,10-15,20,24,25,27. --	20
A	CHEMICAL ABSTRACTS, vol. 115, no. 23, issued 1991, December 09 (Columbus, Ohio, U.S.A.), S. PARMENTIER et al. "Role of glycoprotein IIa (beta1 subunit of very late activation antigens) in platelet functions", page 539, the abstract-no. 252 791h, Blood 1991, 78(8), 2021-6. --	20

* Special categories of cited documents: [14]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11 May 1993	01 -06- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SCHNASS e.h.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 114, no. 9, issued 1991, March 04 (Columbus, Ohio, U.S.A.), R. ONISH et al. "A monoclonal antibody, 2H7, which defines a new very late activation antigen, inhibits IL-2-mediated cell proliferation", page 545, the abstract-no. 79 730s, Nippon Ketsueki Gakkai Zasshi 1990, 53(6), 951-63. --	20
A	CHEMICAL ABSTRACTS, vol. 106, no. 5, issued 1987, February 02 (Columbus, Ohio, U.S.A.), H.G. BLUESTEIN et al. "Immunopathogenesis of the neuropsychiatric manifestations of systematic lupus erythematosus", page 395, the abstract-no. 31 234r, Springer Semin. Immunopathol. 1986, 9(2-3), 237-49. ----	20

INTERNATIONAL SEARCH REPORT

International appli n No.
PCT/US 93/00030

Box I Observations where cer claims were found unsearchable (Continuation of m I of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:

Article 17(2)b) & Rule 39.1(iv) PCT

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/US 93/00030 SAE 68912

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les reseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 330506	30-08-89	EP A3 330506	20-06-90
		JP A2 2003700	09-01-90
WO A1 9200751	23-01-92	AU A1 82055/91	04-02-92
		DK A0 1628/90	06-07-90

P. B. 18 - Patentlaan 2
2280 HV Rijswijk (ZH)
(070) 3 40 20 40
TX 31651 epo nl
FAX (070) 3 40 30 16

Europäisches Patentamt
Zweigstelle in Den Haag Eingangsstelle

European Patent Office
Branch at The Hague Receiving Section

Office européen des brevets
Département à La Haye Section de Dépôt

58 Rec'd PCT/PTO 12 JUL 1994

McNICHOLAS, Janet, M.
Allegretti & Witcoff, Ltd.
Ten South Wacker Drive
Chicago, IL 60606

ETATS-UNIS D'AMERIQUE

Datum/Date
01/09/93

Zeichen/Ref./Réf.	Anmeldung Nr./Application No./Demande n°.//Patent Nr No./Brevet n°.
92,307-A	93902914.6- -PCT/US9300030

Anmelder/Applicant/Demandeur//Patentinhaber/Propriétaire
BIOGEN, INC.

1. European patent application No. 93902914.6 has been allotted to the above-mentioned international patent application.

2. FOR ENTRY INTO THE REGIONAL PHASE BEFORE THE EPO the following procedural steps must be taken:

2.1 Within 21 months from the date of filing or (where applicable) from the earliest priority date if the EPO acts as DESIGNATED OFFICE pursuant to Article 22 PCT:

a) Filing of a translation of the international application in an EPO official language if the International Bureau did not publish the application in one of those languages (Art. 22(1) PCT and Rule 104b(1)(a) EPC).

b) Payment of the national basic fee, the designation fee for each State designated, (where applicable) the claims fees for the eleventh and each subsequent claim and the search fee where a supplementary European search report has to be drawn up (Rule 104b(1)(b), (c) EPC).

2.2 Within 31 months from the date of filing or (where applicable) from the earliest priority date if the EPO acts as ELECTED OFFICE pursuant to Article 39 PCT:

a) Filing of a translation as under 2.1 a)

b) Payment of the fees as under 2.1 b)

c) Filing of the written request for examination and payment of the examination fee (Rule 104b(1)(d) EPC)

d) Payment of the renewal fee for the third year, if due before

--/2

the expiration of the 31 month term (Rule 104b(1)(e) EPC).

3. The amounts of the fees (equivalents in all currencies) are regularly published in the Official Journal of the EPO.

4. If the translation of the international application in an official EPO language is not filed in due time, the international application before the EPO is deemed to be withdrawn (Art. 24(1)(iii), Art. 39(2) PCT).

5. The international search report under Article 18 PCT (or the declaration under Article 17(2) a) PCT) was published on 22.07.93.
This publication takes the place of the mention of the publication of the European search report (Article 157(1) EPC).

A request for examination, comprising a written request and payment of the examination fee, must be filed up to the end of six months after the above date. However, in view of Article 22 or 39 PCT in conjunction with Rule 104b(1)(d) EPC, the period for filing the request for examination does not expire before 21 or 31 months respectively from the date of filing (where applicable, the earliest priority date).

If a request for examination is not filed in due time, the European patent application is deemed to be withdrawn (Art. 94(3) EPC).

6. Applicants and/or representatives having their address within the territory of one of the EPC Contracting States are recommended to file EPO Form 1200 (available free of charge from the EPO) when entering the regional phase.

7. Applicants having neither a residence nor their principal place of business within the territory of one of the EPC Contracting States must be represented by a professional representative whose name appears on the EPO list of representatives (Articles 133(2) and 134(1) EPC).

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No. PCT/US 93/00030

Date stamp of the receiving Office 12 JAN 1993

Applicant's or agent's file reference 92,307-A; D02 CIP PCT

Applicant

BIOGEN, INC.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE	200.00	T	200
2. SEARCH FEE	1,635.00	S	1635

International search to be carried out by EPO

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 44 sheets.

first 30 sheets	525.00	b_1	593
14 remaining sheets x 10.00 additional amount =	140.00	b_2	168
Add amounts entered at b_1 and b_2 and enter total at B	665.00	B	761

Designation Fee

6 number of designations x 127.00 amount of designation fee =	762.00	D	864

(If that total exceeds the figure which corresponds to the amount of the designation fee multiplied by ten, enter the latter figure in box D.)

Add amounts entered at B and D and enter total at I	1,427.00	I	1625
4. FEE FOR PRIORITY DOCUMENT	12.00	P	12
5. TOTAL FEES PAYABLE Add amounts entered at T, S, I and P, and enter total in the TOTAL box	3,274.00 TOTAL		3472

▲ Deleted by IB/US

☐ The designation fee is not paid at this time.

MODE OF PAYMENT

[x]	authorization to charge deposit account (see below)	[ ]	bank draft	[ ]	coupons
[X]	cheque	[ ]	cash	[ ]	other *(specify)*:
[ ]	postal money order	[ ]	revenue stamps		

DEPOSIT ACCOUNT AUTHORIZATION

The RO/ US

[ ] is hereby authorized to charge the total fees indicated above to my deposit account.

[X] is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

[X] is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

01-0850	11 January 1993	Janet M. McNicholas
Deposit Account Number	Date *(day/month/year)*	Signature Janet M. McNicholas

TREATMENT FOR ASTHMA

FIELD OF THE INVENTION

The present invention relates to a treatment for asthma. More particularly, this invention relates to the use of antibodies recognizing Very Late Antigen-4 (VLA-4), a ligand on certain leukocytes for the endothelial cell receptor Vascular Cell Adhesion Molecule-1 (VCAM-1), in the treatment of asthma.

BACKGROUND OF THE INVENTION

Asthma is a condition of the respiratory tract characterized by widespread, reversible narrowing of the airways (bronchoconstriction) and increased sensitivity (hyperresponsiveness) of the airways to a variety of stimuli. The familiar symptomology of asthma, i.e., coughing, wheezing, chest tightness, dyspnea, is caused by airway smooth muscle contraction, increased bronchial mucus secretion, and inflammation. Though seldom fatal, asthma has been estimated to affect 10-20% of school-aged children around the world, and hospital admissions for asthma in children have increased dramatically in recent years, one survey for the United States indicating that hospital admissions for children under 15 with asthma increased by at least 145% between 1970 and 1984. (See, M.R. Sears, 1990 [1].) Overall, it is estimated that 10 million Americans (4% of the population) have asthma, and some $4 billion is spent in treatment per year. (L.K. Altman, 1991 [2]; C. Starr, 1991 [3].)

The causes of asthma are not completely understood, however the study of agents that trigger acute asthmatic episodes supports the theory that asthma is an immunological reaction by a subject in response to specific allergens of the subject's environment. These "triggers" exacerbate asthma by causing transient

corticosteroids such as prednisolone. (See, F.M.C. Cuss, 1990 [5] and P.M. O'Byrne, 1990 [6].)

The inflammatory response in asthma is typical for tissues covered by a mucosa and is characterized by vasodilation, plasma exudation, recruitment of inflammatory cells such as neutrophils, monocytes, macrophages, lymphocytes and eosinophils to the sites of inflammation, and release of inflammatory mediators by resident tissue cells (e.g., mast cells) or by migrating inflammatory cells. (J.C. Hogg, 1990 [7].) In allergen-induced asthma, sufferers often exhibit a dual response to exposure to an allergen --an "early phase" response beginning immediately after exposure and lasting until 1-2 hours after exposure, followed by a "late phase" response beginning about 3 hours after exposure and lasting sometimes until 8-10 hours or longer after exposure. (D.W. Cockroft, 1990 [4].) Late phase response in allergen-induced asthma and persistent hyperresponsiveness have been associated with the recruitment of leukocytes, and particularly eosinophils, to inflamed lung tissue. (W.M. Abraham et al., 1988 [8].) Eosinophils are known to release several inflammatory mediators, e.g., 15-HETE, leukotriene C_4, PAF, cationic proteins, eosinophil peroxidase. (K.F. Chung, 1990 [9].)

Many of the drugs used to treat asthma have been found to block or neutralize the effects of the release of inflammatory mediators which regulate the inflammatory response. For example, $beta_2$-adrenoceptor agonists and DSCG are potent stabilizers of mast cells, which are capable of releasing many mediators, including histamine, prostaglandins, leukotrienes, platelet activating factor (PAF), and chemotactic factors for neutrophils and

and airway hyperresponsiveness in allergic sheep. Surprisingly, administration of anti-VLA-4 led to a reduction in the number of both neutrophils and eosinophils in the lung at 4 hours after allergen challenge, even though both cells have alternate adhesion pathways by which they can be recruited to lung tissues. Also surprisingly, inhibition of hyperresponsiveness in the treated sheep was observed which continued to 1 week, even though infiltration of leukocytes, including neutrophils and eosinophils, was not significantly reduced over time.

SUMMARY OF THE INVENTION

The present invention provides novel methods for the treatment of asthma and further provides new pharmaceutical compositions useful in the treatment of asthma. In particular, the present invention provides a method comprising the step of administering to an asthma sufferer an effective amount of an anti-VLA-4 antibody, such as monoclonal antibody HP1/2. The anti-VLA-4 antibody is advantageously administered *in vivo* to a patient with chronic allergen-induced asthma, and serves to inhibit late phase response to allergens and to attenuate airway hyperresponsiveness.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph depicting the effect of monoclonal antibody HP1/2 (intravenous) on the response to allergen (*Ascaris suum* antigen) in dual responder allergic sheep. Percentage change in specific lung resistance (SR_L) is measured over time post allergen challenge. Asterisks indicate statistically significant results.

eosinophils; corticosteroids, as another example, complex with steroid hormone receptors, which leads to the synthesis of proteins, such as lipocortins, that produce anti-inflammatory effects. (F.M.C. Cuss, 1990 [5].)

Although known asthma medications have some effect on leukocyte recruitment into the lung (W.M. Abraham et al., 1990 [8]), none of these drugs is effective to directly block migration of leukocytes into inflamed tissues.

Inflammatory leukocytes are recruited to sites of inflammation by cell adhesion molecules that are expressed on the surface of endothelial cells and which act as receptors for leukocyte surface proteins or protein complexes. Eosinophils have recently been found to participate in three distinct cell adhesion pathways to vascular endothelium, binding to cells expressing intercellular adhesion molecule-1 (ICAM-1), endothelial cell adhesion molecule-1 (ELAM-1), and vascular cell adhesion molecule-1 (VCAM-1). (P.F. Weller et al., 1991 [10]; G.M. Walsh et al., 1991 [11]; B.S. Bochner et al., 1991 [12]; and A. Dobrina et al., 1991 [13].) VCAM1 binds to the $\alpha_4\beta_1$ integrin, VLA-4, which is expressed on various lymphoid cells, including eosinophils (Weller et al., 1991 [10]; Elices et al. 1990 [14]). That eosinophils express VLA-4 differentiates them from other inflammatory cells such as neutrophils, which bind to ELAM-1 and ICAM-1 but not VCAM-1.

The VLA-4-mediated adhesion pathway was investigated in an asthma model to examine the possible role of VLA-4 in leukocyte recruitment to inflamed lung tissue. It has now been discovered that administering anti-VLA-4 antibody inhibits both the late phase response

8. This information letter is addressed by the EPO to the agent, if any, having acted for the applicant during the international phase of the application. Any future notifications on procedural matters will exclusively be addressed to the applicant respectively his European representative, if the appointment of the latter has been communicated to the EPO in due time.

9. For further details see the Supplement No. 1 to Official Journal No. 12/1992 (Information for PCT applicants concerning time limits and procedural steps before the EPO as a designated and as an elected Office under the PCT (as at 01 January 1993)).

RECEIVING SECTION

enhancement of airway hyperresponsiveness. Triggers that have been found to induce airway hyperresponsiveness include inhaled allergens, inhaled low molecular weight agents to which the subject has become sensitized (e.g., by occupational exposure), viral or mycoplasma respiratory infections, and oxidizing gases such as ozone and nitrogen dioxide. These "inducing" triggers can be distinguished from "inciting" triggers of bronchospastic episodes which include exercise, cold air, emotional stress, pharmacological triggers, inhaled irritants. The common feature of inducing triggers is that they are associated with airways inflammation; inciting triggers produce smooth muscle contractions (bronchospasms) which depend on the underlying degree of hyperresponsiveness, rather than increasing airways responsiveness themselves. (See, D.W. Cockcroft, 1990 [4].)

The recognition that airways inflammation is a cause of transient (acute) and also persistent airway hyperresponsiveness has had an impact on the treatment of asthma sufferers. Early treatments for asthma focused on bronchoconstriction and led to the development of many effective bronchodilator drugs. The most commonly prescribed were $beta_2$-adrenoceptor agonists (epinephrine, isoproterenol, albuterol, salmeterol, etc.), xanthines (caffeine, theophylline, etc.) and cholinoceptor antagonists (atropine, acetylcholine, etc.). More recently, however, anti-inflammatory drugs have begun to replace bronchodilators as first-line treatments for asthma. Commonly prescribed anti-inflammatory agents for asthma include disodium cromoglycate (DSCG), nedocromil sodium, antihistamines such as ketotifen, and

Figure 2 is a graph depicting plasma concentration of monoclonal antibody HP1/2 (intravenous) in sheep, measured over time after initial administration.

Figure 3 is a graph depicting the effect of monoclonal antibody HP1/2 (intravenous) on airway hyperresponsiveness in dual responder sheep. Airway responsiveness, measured in breath units (BU) of cumulative breaths of a 1% weight/volume carbachol solution (a known bronchoconstrictor) that increases specific lung resistance 400% over the value obtained using diluent alone. Asterisks indicate statistically significant results.

Figure 4 is a series of four graphs showing the total cells and the levels of different leukocytes (lymphocytes, neutrophils, and eosinophils) detected by bronchoalveolar lavage in allergic sheep challenged with Ascaris suum antigen alone and after pretreatment with monoclonal antibody HP1/2 (intravenous). Total cells, and the percentage of total cells that were lymphocytes or neutrophils or eosinophils, were measured at 4-hour, 8-hour, 24-hour, 48-hour and 1-week time points post allergen challenge.

Figure 5 is a graph depicting the effect of monoclonal antibody HP1/2 (16 mg, aerosol) and 1E6 (16 mg, aerosol) on the response to allergen (Ascaris suum antigen) in dual responder allergic sheep. Percentage change in specific lung resistance (SR_L) is measured over time post allergen challenge. Asterisks indicate statistically significant results.

Figure 6 is a graph depicting the effect of monoclonal antibody HP1/2 (16 mg, aerosol) and 1E6 (16 mg, aerosol) on airway hyperresponsiveness in dual responder

sheep. Airway responsiveness, measured in breath units (BU) of cumulative breaths of a 1% weight/volume carbachol solution (a known bronchoconstrictor) that increases specific lung resistance 400% over the value obtained using diluent alone. Asterisks indicate statistically significant results.

DETAILED DESCRIPTION OF THE INVENTION

The technology for producing monoclonal antibodies is well known. Briefly, an immortal cell line (typically myeloma cells) is fused to lymphocytes (typically splenocytes) from a mammal immunized with whole cells expressing a given antigen, e.g., VLA-4, and the culture supernatants of the resulting hybridoma cells are screened for antibodies against the antigen. (See, generally, Kohler et al., 1975 [15].)

Immunization may be accomplished using standard procedures. The unit dose and immunization regimen depend on the species of mammal immunized, its immune status, the body weight of the mammal, etc. Typically, the immunized mammals are bled and the serum from each blood sample is assayed for particular antibodies using appropriate screening assays. For example, anti-VLA-4 antibodies may be identified by immunoprecipitation of ^{125}I-labeled cell lysates from VLA-4-expressing cells. (See, Sanchez-Madrid et al. 1986 [16] and Hemler et al. 1987 [17].) Anti-VLA-4 antibodies may also be identified by flow cytometry, e.g., by measuring flourescent staining of Ramos cells incubated with an antibody believed to recognize VLA-4 (see, Elices et al., (1990) [14]). The lymphocytes used in the production of hybridoma cells typically are isolated from immunized mammals whose sera have already tested positive

for the presence of anti-VLA-4 antibodies using such screening assays.

Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium").

Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using 1500 molecular weight polyethylene glycol ("PEG 1500"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridomas producing a desired antibody are detected by screening the hybridoma culture supernatants. For example, hybridomas prepared to produce anti-VLA-4 antibodies may be screened by testing the hybridoma culture supernatant for secreted antibodies having the ability to bind to a recombinant α_4-subunit-expressing cell line, such as transfected K-562 cells (see, Elices et al. [14]).

To produce anti VLA-4-antibodies, hybridoma cells that tested positive in such screening assays were cultured in a nutrient medium under conditions and for a time sufficient to allow the hybridoma cells to secrete the monoclonal antibodies into the culture medium. Tissue culture techniques and culture media suitable for hybridoma cells are well known. The conditioned hybridoma culture supernatant may be collected and the anti-VLA-4 antibodies optionally further purified by well-known methods.

Alternatively, the desired antibody may be produced by injecting the hybridoma cells into the peritoneal cavity of an unimmunized mouse. The hybridoma cells proliferate in the peritoneal cavity, secreting the antibody which accumulates as ascites fluid. The antibody may be harvested by withdrawing the ascites fluid from the peritoneal cavity with a syringe.

Several anti-VLA-4 monoclonal antibodies have been previously described (see, e.g., Sanchez-Madrid et al., 1986 [16]; Hemler et al. (1987) [17]; Pulido et al. (1991) [19]). For the experiments herein, an anti-VLA-4 monoclonal antibody designated HP1/2 (obtained from Biogen, Inc., Cambridge, MA) was used. The variable regions of the heavy and light chains of the anti-VLA-4 antibody HP1/2 have been cloned, sequenced and expressed in combination with constant regions of human immunoglobulin heavy and light chains. Such a chimeric HP1/2 antibody is similar in specificity and potency to the murine HP1/2 antibody, and may be useful in methods of treatment according to the present invention. Similarly, humanized recombinant anti-VLA-4 antibodies may be useful in these methods. The HP1/2 V_H DNA sequence and its translated amino acid sequences are set forth in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The HP1/2 V_K DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

Monoclonal antibodies such as HP1/2 and other anti-VLA-4 antibodies (e.g., Mab HP2/1, HP2/4, L25, P4C2) capable of recognizing the α chain of VLA-4 will be useful in the present invention. It is most preferred that the antibodies will recognize the B1 or B2 epitopes of the VLA-α_4 chain (see, Pulido et al. (1991) [19]). While not

wishing to be bound by one scientific theory, anti-VLA-4 antibodies used according to the method of the present invention may specifically inhibit, at least for an initial period following allergen challenge, the migration of VLA-4-expressing leukocytes to inflamed sections of the lung. This inhibition of VLA-4 leukocyte migration could, in turn, prevent secondary pathological effects of leukocyte infiltration, e.g., release of toxic substances, inducement of soluble inflammatory cell mediators, release or inducement of leukocyte chemotactic agents (such as neutrophil chemotactic factors), etc. As a result, late phase response to the allergen and continuing hypersensitivity of the airways may be attenuated. Alternatively, the anti-VLA-4 antibodies may attenuate signal transduction necessary for the release of inflammatory mediators and/or cell chemotactic agents.

The method of the present invention comprises administering to a mammal suffering from allergic asthma a composition comprising an anti-VLA-4 antibody. The examples below set forth the results observed in asthmatic sheep. However, the similarity between physiological responses and pharmacological effects in sheep and in humans has been documented (see, e.g., W.M. Abraham, 1989 [20]); and similarities between sheep and other animal asthma models (rabbits, squirrel monkeys, guinea pigs, and sensitized dogs) have been noted (see, e.g., W.M. Abraham et al., 1988 [8]). Accordingly, the results reported herein will be relevant and applicable to, and the method claimed will be useful in, any mammal, including humans, suffering from allergic asthma.

The anti-VLA-4 antibody administered in accordance with the present invention may be administered prophylactically, before exposure to an asthma-inducing

allergen. Beneficial effects will also be obtained if the antibody is administered at the time of or immediately after allergen exposure, between early phase and late phase response to attenuate the severity of late phase response, or at any time following allergen exposure to reduce or eliminate airway hyperresponsiveness.

The anti-VLA-4 antibody can be administered in the form of a composition comprising an anti-VLA-4 antibody and a pharmaceutically acceptable carrier. Preferably, the composition will be in a form suitable for intravenous injection. Also contemplated are antibody compositions in the form of a sterile aqueous or phosphate-buffered saline solution which can be nebulized (atomized) and breathed directly into the lungs by the asthma sufferer, e.g., using an inhaler. Dosages will vary depending on the sensitivity of the asthma sufferer to particular allergens, the concentration of allergen on exposure and frequency/duration of exposure(s), the proposed mode of administration (e.g., injection or inhalation), the desired plasma level of antibody, the effectiveness of a particular antibody or combination of antibodies in suppressing airway responsiveness, the clearance rate or half-life of the antibody composition, and other such factors familiar to physicians experienced in the treatment of allergic asthma. In general, dosages will be calculated and adjusted to maintain a plasma level of antibody in the range of from 1-1000 μg/ml, although higher or lower dosages may be indicated with consideration to the age, sensitivity, tolerance, and other characteristics of the patient, the acuteness of the flareup, the history and course of the disease, and other similar factors routinely considered by an attending physician. Depending on the potency and half-life of the

antibody employed, it is preferred to use from about 0.05 mg/kg to 5.0 mg/kg of antibody, most preferably from 0.5 to 2.0 mg/kg of antibody, based on the weight of the patient receiving treatment.

Suitable pharmaceutical carriers include, e.g., sterile saline and physiological buffer solutions. Phosphate buffered saline (PBS) is preferred for inhalant administration. The pharmaceutical compositions may additionally be formulated to control the release of the active ingredients or to prolong their presence in the patient's system. Numerous suitable drug delivery systems are known for this purpose and include, e.g., hydrogels, hydroxmethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like.

It will also be recognized that for the purposes of the present invention, antibodies capable of binding to the α_4 subunit of VLA-4 must be employed. It is preferred that monoclonal antibodies be used.

In addition to naturally produced antibodies, suitable recombinant antibodies capable of binding to VLA-4 may alternatively be used. Such recombinant antibodies include antibodies produced via recombinant DNA techniques, e.g., by transforming a host cell with a suitable expression vector containing DNA encoding the light and heavy immunoglobulin chains of the desired antibody, and recombinant chimeric antibodies, wherein some or all of the hinge and constant regions of the heavy and/or the light chain of the anti-VLA-4 antibody have been substituted with corresponding regions of an immunoglobulin light or heavy chain of a different species (i.e., preferably the same species as the asthma sufferer being treated, to minimize immune response to the

administered antibody). (See, e.g., P.T. Jones et al., 1986 [21], E.S. Ward et al., 1989 [22], and U.S. Patent 4,816,397 (Boss et al.) [23], all incorporated herein by reference.)

Furthermore, VLA-4-binding fragments of anti-VLA-4 antibodies, such as Fab, Fab', $F(ab')_2$, and F(v) fragments; heavy chain monomers or dimers; light chain monomers or dimers; and dimers consisting of one heavy chain and one light chain are also contemplated herein. Such antibody fragments may be produced by chemical methods, e.g., by cleaving an intact antibody with a protease, such as pepsin or papain, or via recombinant DNA techniques, e.g., by using host cells transformed with truncated heavy and/or light chain genes. Heavy and light chain monomers may similarly be produced by treating an intact antibody with a reducing agent such as dithiothreitol or β-mercaptoethanol or by using host cells transformed with DNA encoding either the desired heavy chain or light chain or both.

Also, from the foregoing discussion it will be apparent that other polypeptides and molecules which inhibit or block VLA-4-mediated binding will be effective in the treatment of asthma in the same manner as anti-VLA-4 antibodies. For example, a soluble form of VCAM-1 (an endothelial cell receptor for VLA-4) or a fragment thereof may be administered to compete for the VLA-4 binding site, thereby leading to effects similar to the administration of anti-VLA-4 antibodies. Small molecules such as oligosaccharides that mimic the binding domain of an VLA-4 ligand and fit the receptor domain of VLA-4 may also be employed. (See, J.J. Devlin et al., 1990 [24], J.K. Scott and G.P. Smith, 1990 [25], and U.S. Patent 4,833,092

(Geysen) [26], all incorporated herein by reference.) The use of such VLA-4-binding polypeptides or molecules that effectively inhibit late phase response or airway hyperresponsiveness in allergic subjects is contemplated herein as an alternative method for treatment of asthma.

It is also contemplated that anti-VLA-4 antibodies may be used in combination with other antibodies having a therapeutic effect on airway responsiveness. For instance, to the extent that the beneficial effects reported herein are due to the inhibition of leukocyte recruitment to VCAM-1-expressing endothelium, combinations of anti-VLA-4 antibodies with other antibodies that interfere with the adhesion between leukocyte antigens and endothelial cell receptor molecules may be advantageous. For example, in addition to the use of anti-VLA-4 antibodies in accordance with this invention, the use of anti-ELAM-1 and/or anti-ICAM-1 antibodies may be advantageous. [See, Gundel et al. (1991) [27]; Wegner et al. (1990) [28].)

When formulated in the appropriate vehicle, the pharmaceutical compositions contemplated herein may be administered by any suitable means such as orally, intraesophageally or intranasally, intrabronchially (local treatment, e.g., via bronchoscope), as well as subcutaneously, intramuscularly, intravenously, intra-arterially, or parenterally. Ordinarily administration via inhalation is preferred.

EXAMPLES

Experiments were performed essentially as described by Abraham et al. [8]. Briefly, allergic sheep having natural allergic cutaneous reaction to 1:1000 or 1:10,000 dilutions of Ascaris suum extract (Greer Diagnostics, Lenoir NC) were tested, and sheep

demonstrating both early and late phase airway response ("dual responders") to inhalation challenge with Ascaris suum antigen were selected. To measure breathing mechanics and physical changes in the airways, the sheep were restrained in a prone position with heads immobilized. A balloon catheter was advanced through one nostril under topical anesthesia with 2% lidocaine solution to the lower esophagus, and a cuffed endotracheal tube was advanced through the other nostril (using a flexible fiberoptic bronchoscope as a guide) for the measurement of airway mechanics and during aerosol challenges. Pleural pressure was estimated with the esophageal balloon catheter (filled with 1 ml of air) positioned 5-10 cm from the gastroesophageal junction. In this position, end expiratory pleural pressure ranged between -2 and -5 cm H_2O. Once the balloon was placed, it was secured so that it remained in position for the duration of the experiment. Lateral pressure in the trachea was measured with a sidehole catheter, (inner diam. 2.5 mm) advanced through and positioned distal to the tip of thee endotracheal tube. Transpulmonary pressure (the difference between tracheal and pleural pressure) was measured with a differential pressure transducer catheter system (MP45, Validyne, Northridge, CA). The pressure transducer catheter system showed no phase shift between pressure and flow to a frequency of 9 H_Z. Pulmonary resistance (R_L) was measured by connecting the proximal end of the endotracheal tube to a Fleich pneumotachograph (Dyna Sciences, Blue Bell PA). Signals indicating flow and transpulmonary pressure were recorded on an oscilloscope recorder (Model DR-12; Electronics for Medicine, White Plains, NY) linked to a

computer for automatic calculation of pulmonary resistance (R_L) from transpulmonary pressure, respiratory volume (obtained by digital integration) and flow by the mid-volume technique, analyzed from 5-10 breaths. Thoracic gas volume (V_{tg}) was measured immediately after determination of R_L in a constant volume body plethysmograph. Specific lung resistance (SR_L) was calculated from these values ($SR_L = V_{tg} \times R_L$).

Airway responsiveness was determined by performing dose response curves to inhaled carbachol. The dose response curves were plotted using measurements of SR_L taken immediately after inhalation of buffer (PBS) alone and after each consecutive administration of 10 breaths of increasing concentrations of carbachol in PBS. The concentrations of carbachol were 0.25%, 0.5%, 1.0%, 2.0% and 4.0% wt/vol in PBS. The provocation test was discontinued when SR_L increased over 400% from the post-PBS value or after the highest carbachol concentration had been administered. Airway responsiveness was determined by calculating from the dose response curves the cumulative carbachol dose in breath units (BU) that increased specific lung resistance 400% over the post buffer value ($PD_{400\%}$). One breath unit was defined as one breath of a 1% wt/vol carbachol solution. Thus, the greater the suppression of airway hyper-responsiveness, the greater the number of breath units would be required before observing the same bronchoconstriction as seen in the controls.

Each sheep was subjected to a trial as a control in which a placebo (PBS without additive) was used as a pretreatment 30 minutes before allergen challenge with Ascaris suum antigen (Greer Diagnostics, Lenoir, NC).

Subsequently, the sheep were subjected to an identical trial, except that 1 mg/kg of monoclonal antibody HP1/2 was administered to each sheep 30 minutes prior to antigen challenge. The placebo (buffer control or isotope-matched antibody (1E6, anti-LFA3) control) and HP1/2 compositions were administered by intravenous injection. The HP1/2 composition (and the 1E6 control) was prepared by diluting pure antibody obtained from a hybridoma (Biogen, Inc., Cambridge MA) in sterile, endotoxin-free PBS and adjusting to deliver 1 mg/kg antibody based on the weight of each sheep. The antigen solution was delivered as an aerosol using a disposable medical nebulizer (RAINDROP®, Puritan Bennett, Lenexa, KS) that provided an aerosol with a mass median aerodynamic diameter of 3.2 μM (geometric SD 1.9) as determined by an Andersen cascade impactor. The Ascaris suum extract was diluted in PBS to a concentration of 82,000 Protein Nitrogen Units(PNU)/ml. The output of the nebulizer was directed into a plastic T-tube, one end of which was connected to the inspiratory port of a Harvard respirator. A dosimeter connected to the nebulizer consisting of a solenoid valve and a 20 psi compressed air source and the solenoid valve was activated at the beginning of the inspiratory cycle of the Harvard respirator for one second. The aerosol delivered at a tidal volume of 500 ml and a rate of 20 breaths per min. for 20 min. Each sheep was challenged with an equivalent dose of antigen (400 breaths) in the control and HP1/2 trials. Carbachol aerosols for the dose response curves were also generated by nebulizer as described above.

For cellular analysis, bronchoalveolar lavage (BAL) was performed on each sheep. The distal tip of the specially designed 80 cm fiberoptic bronchoscope was gently wedged into a randomly selected subsegmental

bronchus. Lung lavage was performed by slow infusion and gentle aspiration of 3 x 30 ml of PBS (pH 7.4) at 39° C, using 30 ml syringes attached to the working channel of the bronchoscope. The lavage return was collected, strained through gauze to remove mucus and then centrifuged at 420 g for 15 min. Supernatant was decanted, and the cells were resuspended in PBS. An aliquot of the suspension was transferred to a hemocytometer chamber to estimate total cells. Total viable cells were estimated by trypan blue exclusion. A second aliquot of the cell suspension was spun in a cytospin (600 rpm for 10 minutes) and stained by Wright-Giemsa and observed at 100X to identify cell populations. 500 cells per slide were characterized to establish the differential cell counts. Cells characterized included epithelial cells, macrophages, basophils, monocytes and unidentifiable cells (grouped into a category termed "others"), in addition to lymphocytes, neutrophils and eosinophils.

Plasma level of antibody and white blood cell counts were determined from venous blood samples (approx. 5 ml) from peripheral leg vein or jugular vein.

Example 1

An airway challenge trial using eight dual responder allergic sheep was conducted according to the foregoing protocols. Baseline (BSL) airway responsiveness ($PD_{400\%}$) was established 2-3 days prior to antigen challenge and a baseline bronchoalveolar lavage (BAL) was performed one day prior to challenge. On challenge day, baseline values for specific lung resistance (SR_L) was measured, then the sheep were administered buffer (control) or HP1/2 by injection. After this first

administration ("treatment"), SR_L was measured, and 30 min. after treatment, the sheep were challenged with Ascaris suum antigen. SR_L was measured immediately after challenge, hourly from 1-6 hours following challenge, every half-hour from 6.5 hours to 8 hours, and also at 24 hours, 48 hours and 1 week (i.e., 168 hours) after antigen challenge. BALs were performed following SR_L measurements at 4, 8, 24 and 48 hours and at 1 week. For these studies, peripheral blood was drawn and total white blood cell counts and assessment of cell populations were taken before treatment (baseline), immediately after challenge, and at 1, 2, 3, 4, 6, 8, 24 and 48 hours, and 1 week after challenge. The results of this trial are shown in the figures:

Figure 1 shows the effect of HP1/2 treatment on antigen-induced airway responses in the subject sheep. HP1/2 treatment resulted in significant (indeed, virtually complete) inhibition of the late phase response experienced by the controls.

Figure 2 is a graph of plasma concentration of HP1/2 in μg/ml in the treated subjects, measured immediately following antigen challenge and then at 1, 2, 3, 4, 6, 8, 24 and 48 hours after challenge. After equilibration, the antibody concentration settled to a concentration of approximately 20 μg/ml, which concentration was maintained out to the 48-hour point.

Figure 3 is a graph showing the effect of HP1/2 treatment on airway responsiveness. At 24, 48, and 1 week after antigen challenge, treated subjects showed significant decrease in airway responsiveness. Even at 2 weeks after antigen challenge, treated subjects continued to show decreases in airway responsiveness. The fact that

the virtually complete inhibitory effect of the antibody lasted out to 1 week is especially surprising and encouraging in terms of the therapeutic value of the treatment.

Figure 4 is a series of graphs illustrating the results of BALs performed at 4, 8, 24 and 48 hours after antigen challenge, and at 1 week after antigen challenge. The results show no significant changes over controls in total cells recovered from treated subjects. However, treated subjects showed reduced levels of both neutrophils and eosinophils at the 4-hour time point after challenge. This is somewhat surprising, given that the administration of anti-VLA-4 would not be expected to influence neutrophil recruitment, since neutrophils do not express VLA-4. Also, both neutrophils and eosinophils express alternative ligands involved in adhesion to endothelium; both types of cells have been shown to bind to endothelial cells via the LFA-1/ICAM-1 pathway and the CDX/ELAM-1 pathway.

Similar therapeutic effects with the anti-VLA-4 antibody HP1/2 were observed when the subjects were treated with HP1/2 antibody 2 hours after antigen challenge as opposed to 30 minutes prior to challenge as described above. The effect of HP1/2 was dose-dependent. For example, reducing the dose to 0.2 mg/kg was not sufficient to protect against the late response. For the antigen challenge studies in which 1E6 (anti-LFA3) was used as an isotope-matched control antibody for the HP1/2 treatment, no effect on the early or late response was observed using 1E6 in a control trial. The 1E6-2C12 hybridoma cell line producing the 1E6 antibody has been deposited as ATCC HB 10693.

is specific because the same dose of 1E6 had no protective effect (e.g., 1E6 treated animals showed a significant fall in PC_{400}, whereas HP1/2 blocked the effect). The differences in the physiological responses between HP1/2 and 1E6 are not the result of deficiencies in total WBC or differential counts between the groups. Total WBC and differential in both the HP1/2 and 1E6 groups showed a pattern of responses similar to those seen in the intravenous trials.

The foregoing examples are intended as an illustration of the method of the present invention and are not presented as a limitation of the invention as claimed hereinafter. From the foregoing disclosure, numerous modifications and additional embodiments of the invention will be apparent to those experienced in this art. For example, actual dosage used, the type of antibody or antibody fragment used, mode of administration, exact composition, time and manner of administration of the treatment, and many other features all may be varied without departing from the description above. All such modifications and additional embodiments are within the contemplation of this application and within the scope of the appended claims.

CITED PUBLICATIONS

[1] M. R. Sears, "Epidemiology of Asthma," in Asthma as an Inflammatory Disease, P. O'Byrne, ed. (Marcel Dekker, Inc.; New York 1990), pp. 15-48

[2] L. K. Altman, "Despite Gains in Treatment, Asthma Worsens," New York Times, The Doctor's World, March 26, 1991

[3] C. Starr, "Treating Asthma: A Killer Gathers Strength," Drug Topics, cover story, issue of April 8, 1991

[4] D. W. Cockcroft, "Atopy and Asthma," in Asthma as an Inflammatory Disease, P. O'Byrne, ed. (Marcel Dekker, Inc.; New York 1990), pp. 103-125

[5] F. M. C. Cuss, "The Pharmacology of Antiasthma Medications," in Asthma as an Inflammatory Disease, P. O'Byrne, ed. (Marcel Dekker, Inc.; New York 1990), pp. 199-250

[6] P. M. O'Byrne, "Airway Inflammation and Asthma," in Asthma as an Inflammatory Disease, P. O'Byrne, ed. (Marcel Dekker, Inc.; New York 1990), pp. 143-157

[7] J. C. Hogg, "Pathology of Asthma," in Asthma as an Inflammatory Disease, P. O'Byrne, ed. (Marcel Dekker, Inc.; New York 1990), pp. 1-13

[8] W. M. Abraham et al., "Cellular Markers of Inflammation in the Airways of Allergic Sheep with and without Allergen-induced Late Responses," Am. Rev. Respir. Dis., 138, 1565-1571 (1988)

[9] K. F. Chung, "Inflammatory Mediators in Asthma," in Asthma as an Inflammatory Disease, P. O'Byrne, ed. (Marcel Dekker, Inc.; New York 1990), pp. 159-183

[10] P. F. Weller et al., "Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1," Proc. Natl. Acad. Sci. USA, 88, 7430-7433 (1991)

[11] G. M. Walsh et al., "Human Eosinophil, But Not Neutrophil, Adherence to IL-1-Stimulated Human Umbilical Vascular Endothelial Cells Is $\alpha_4\beta_1$ (Very Late Antigen-4) Dependent," J. Immunol., 146, 3419-3423 (1991)

[12] B. S. Bochner et al., "Adhesion of Human Basophils, Eosinophils, and Neutrophils to Interleukin 1-activated Human Vascular Endothelial Cells: Contributions of Endothelial Cell Adhesion Molecules," J. Exp. Med., 173, 1553-1556 (1991)

[13] A. Dobrina et al., "Mechanisms of Eosinophil Adherence to Cultured Vascular Endothelial Cells," J. Clin. Invest., 88, 20-26 (1991)

[14] M. J. Elices et al., "VCAM-1 on Activated Endothelium Interacts with the Leukocyte Integrin VLA-4 at a Site Distinct from the VLA-4/Fibronectin Binding Site," Cell, 60, 577-584 (1990)

[15] Kohler and Milstein, "Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity," Nature, 256, pp. 495-7 (1975)

[16] F. Sanchez-Madrid et al., "VLA-3: A novel polypeptide association within the VLA molecular complex: cell distribution and biochemical characterization," Eur. J. Immunol., 16, 1343-1349 (1986)

[17] M. E. Hemler et al., "Characterization of the Cell Surface Heterodimer VLA-4 and Related Peptides," J. Biol. Chem., 262(24), 11478-11485 (1987)

[18] L. Osborn et al., "Direct cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes," Cell, 59, 1203-1211 (1989)

[19] R. Pulido et al., "Functional Evidence for Three Distinct and Independently Inhibitable Adhesion Activities Mediated by the Human Integrin VLA-4," J. Biol. Chem., 266(16), 10241-10245 (1991)

[20] W. M. Abraham, "Pharmacology of Allergen-Induced Early and Late Airway Responses and Antigen-Induced

Airway Hyperresponsiveness in Allergic Sheep," Pulmonary Pharmacology, 2, pp. 33-40 (1989)

[21] P. T. Jones et al., "Replacing the Complementarity-Determining Regions in a Human Antibody with Those From a Mouse", Nature, 321, pp. 522-525 (1986)

[22] E. S. Ward et al., "Binding Activities of a Repertoire of Single Immunoglobulin Variable Domains Secreted From Escherichia coli", Nature, 341, pp. 544-546 (1989).

[23] U.S. Patent No. 4,816,397, Boss et al., "Multichain Polypeptides Or Proteins And Processes For Their Production", March 28, 1989.

[24] J. J. Devlin et al., "Random Peptide Libraries: A Source of Specific Protein Binding Molecules", Science, 249, pp. 40-406 (1990)

[25] J. K. Scott and G. P. Smith, "Searching for Peptide Ligands with an Epitope Library", Science, 249, pp. 386-390 (1990)

[26] U.S. Patent No. 4,833,092, Geysen, "Method For Determining Mimotopes", May 23, 1989.

[27] R. H. Gundel et al., "Endothelial Leukocyte Adhesion Molecule-1 Mediates Antigen-induced Acute Airway Inflammation and Late-phase Airway Obstruction in Monkeys," J. Clin. Invest., 88, 1407-1411 (1991)

[28] C. D. Wegner et al., "Intercellular Adhesion Molecule-1 (ICAM-1) in the Pathogenesis of Asthma," Science, 247, 456-459 (1990)

The foregoing documents are incorporated herein by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lobb, Roy R.

(ii) TITLE OF INVENTION: Treatment for Asthma

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Allegretti & Witcoff, Ltd.
(B) STREET: 10 South Wacker Drive, Suite 3000
(C) CITY: Chicago
(D) STATE: IL
(E) COUNTRY: US
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT
(B) FILING DATE: 12 January 1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: McNicholas, Janet M.
(B) REGISTRATION NUMBER: 32,918
(C) REFERENCE/DOCKET NUMBER: 92,307-A; D002 CIP PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312-715-1000
(B) TELEFAX: 312-715-1234

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG159 insert: HP1/2 heavy chain variable region; amino acid 1 is Glu (E) but Gln (Q) may be substituted"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GTC AAA CTG CAG CAG TCT GGG GCA GAG CTT GTG AAG CCA GGG GCC TCA        48
Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser
 2                  6                  11                  16

GTC AAG TTG TCC TGC ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT        96
Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr
            21                  26                  31

ATG CAC TGG GTG AAG CAG AGG CCT GAA CAG GGC CTG GAG TGG ATT GGA       144
Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
        36                  41                  46

AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC CCG AAG TTC CAG       192
Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln
    51                  56                  61

GTC AAG GCC ACT ATT ACA GCG GAC ACG TCC TCC AAC ACA GCC TGG CTG       240
Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
66                  71                  76                  81

CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT GCC GTC TAC TAC TGT GCA       288
Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                86                  91                  96

GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC TTC TGG GGC CAA       336
Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln
            101                 106                 111

GGG ACC ACG GTC ACC GTC TCC TCA                                      360
Gly Thr Thr Val Thr Val Ser Ser
        116                 121
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser
  2                   6                  11                  16

Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr
              21                  26                  31

Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
          36                  41                  46

Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln
      51                  56                  61

Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
  66                  71                  76                  81

Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                  86                  91                  96

Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln
              101                 106                 111

Gly Thr Thr Val Thr Val Ser Ser
          116                 121
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 318 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..318
(D) OTHER INFORMATION: /product= "HP1/2 light chain variable region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG172 insert: HP1/2 light chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTT TCA GCA GGA          48
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
  1               5                  10                  15

GAC AGG GTT ACC ATA ACC TGC AAG GCC AGT CAG AGT GTG ACT AAT GAT          96
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
             20                  25                  30

GTA GCT TGG TAC CAA CAG AAG CCA GGG CAG TCT CCT AAA CTG CTG ATA         144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
         35                  40                  45

TAT TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT CGC TTC ACT GGC         192
Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
     50                  55                  60

AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG GCT         240
Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
 65                  70                  75                  80

GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCG TAC         288
Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr
                 85                  90                  95

ACG TTC GGA GGG GGG ACC AAG CTG GAG ATC                                318
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
  1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
             20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
         35                  40                  45
```

Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
65 70 75 80

Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105

CLAIMS:

1. A method for the treatment of asthma comprising administering to a mammal suffering from asthma a composition comprising an anti-VLA-4 antibody.

2. The method of Claim 1, wherein the anti-VLA-4 antibody composition is administered intravenously.

3. The method of Claim 1, wherein the anti-VLA-4 antibody composition is administered in the form of an aerosol by inhalation.

4. The method of Claim 1, wherein the anti-VLA-4 antibody is selected from HP1/2, HP2/1, HP2/4, L25, and P4C2.

5. The method of Claim 1, wherein the anti-VLA-4 antibody is HP1/2, or a fragment thereof capable of binding to VLA-4.

6. The method of Claim 1, wherein the composition is administered at a dosage so as to provide from 0.05 to 5.0 mg/kg of antibody, based on the weight of the asthma sufferer.

7. The method of Claim 6, wherein the composition is administered at a dosage so as to provide 0.5 to 2.0 mg/kg of antibody, based on the weight of the asthma sufferer.

8. The method according to Claim 1, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the mammal of at least 10 μg/ml.

9. The method of Claim 1, wherein the composition is administered prior to exposure to an allergen to which the asthma sufferer is hypersensitive.

10. The method of Claim 1, wherein the mammal is a human.

11. The method of Claim 1, wherein the composition is administered after exposure to an allergen to which said mammal is hypersensitive.

12. A method for the treatment of asthma comprising administering to a mammal suffering from allergic asthma an antibody, a recombinant antibody, a chimeric antibody, fragments of such antibodies, a polypeptide or a small molecule capable of binding to the α_4 subunit of VLA-4, or combinations of any of the foregoing, in an amount effective to provide inhibition of late phase response to an allergen to which the sufferer is hypersensitive or to provide decreased airway hypersensitivity in said mammal following allergen challenge.

13. The method of Claim 12, wherein the antibody, polypeptide or molecule is selected from monoclonal antibody HP1/2; Fab, Fab', $F(ab')_2$ or F(v) fragments of such antibody; soluble VCAM-1 polypeptides; or small molecules that bind to the VCAM-1-binding domain of VLA-4.

14. The method of Claim 12, wherein the composition comprises a plurality of anti-VLA-4 monoclonal antibodies or VLA-4-binding fragments thereof.

15. The method of Claim 12, wherein the composition includes, in addition to anti-VLA-4, an anti-ELAM-1 antibody, or an anti-ICAM-1 antibody, or both anti-ELAM-1 and anti-ICAM-1 antibodies.

16. The method of Claim 12, wherein the anti-VLA-4 antibody is HP1/2, or a fragment thereof capable of binding to VLA-4.

17. The method of Claim 12, wherein the composition is administered at a dosage so as to provide

from 0.05 to 5.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

18. The method of Claim 17, wherein the composition is administered at a dosage so as to provide 1.0-2.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

19. The method according to Claim 12, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the mammal of at least 10 μg/ml over a period of 7 days.

20. A pharmaceutical composition effective to attenuate late phase response or significantly reduce airway hypersensitivity in an asthmatic mammal consisting essentially of a monoclonal antibody recognizing VLA-4 in a pharmaceutically acceptable carrier.

Abstract of the Disclosure

A method for the treatment of asthma is disclosed. The method comprises administration of an antibody, polypeptide or other molecule recognizing VLA-4, a protein expressed on the surface of certain leukocytes such as eosinophils.

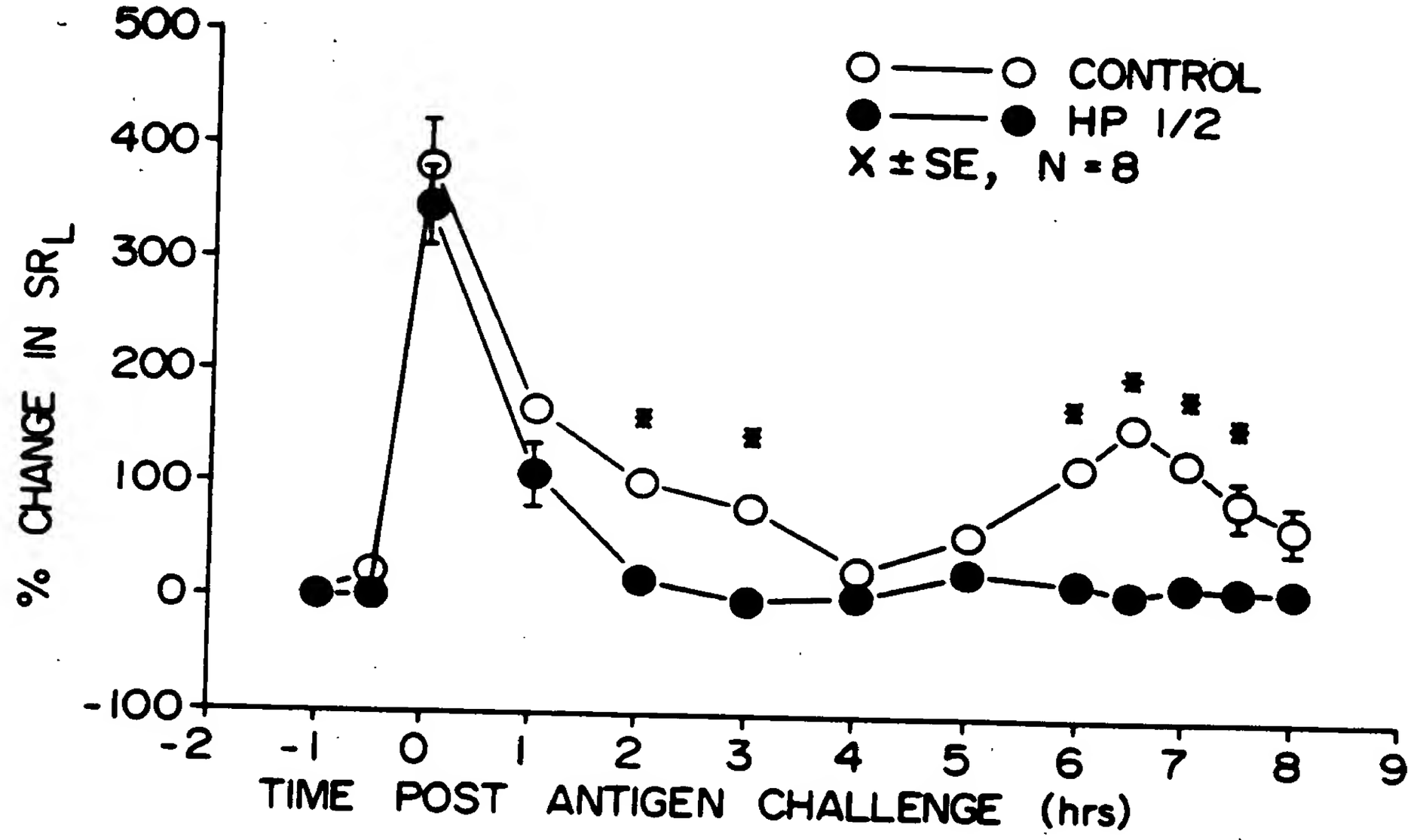

CONTROL
HP 1/2
X ± SE, N = 8
% CHANGE IN SRL
500
400
300
200
100
0
-100
-2
-1
0
1
2
3
4
5
6
7
8
9
TIME POST ANTIGEN CHALLENGE (hrs)

FIG. 1

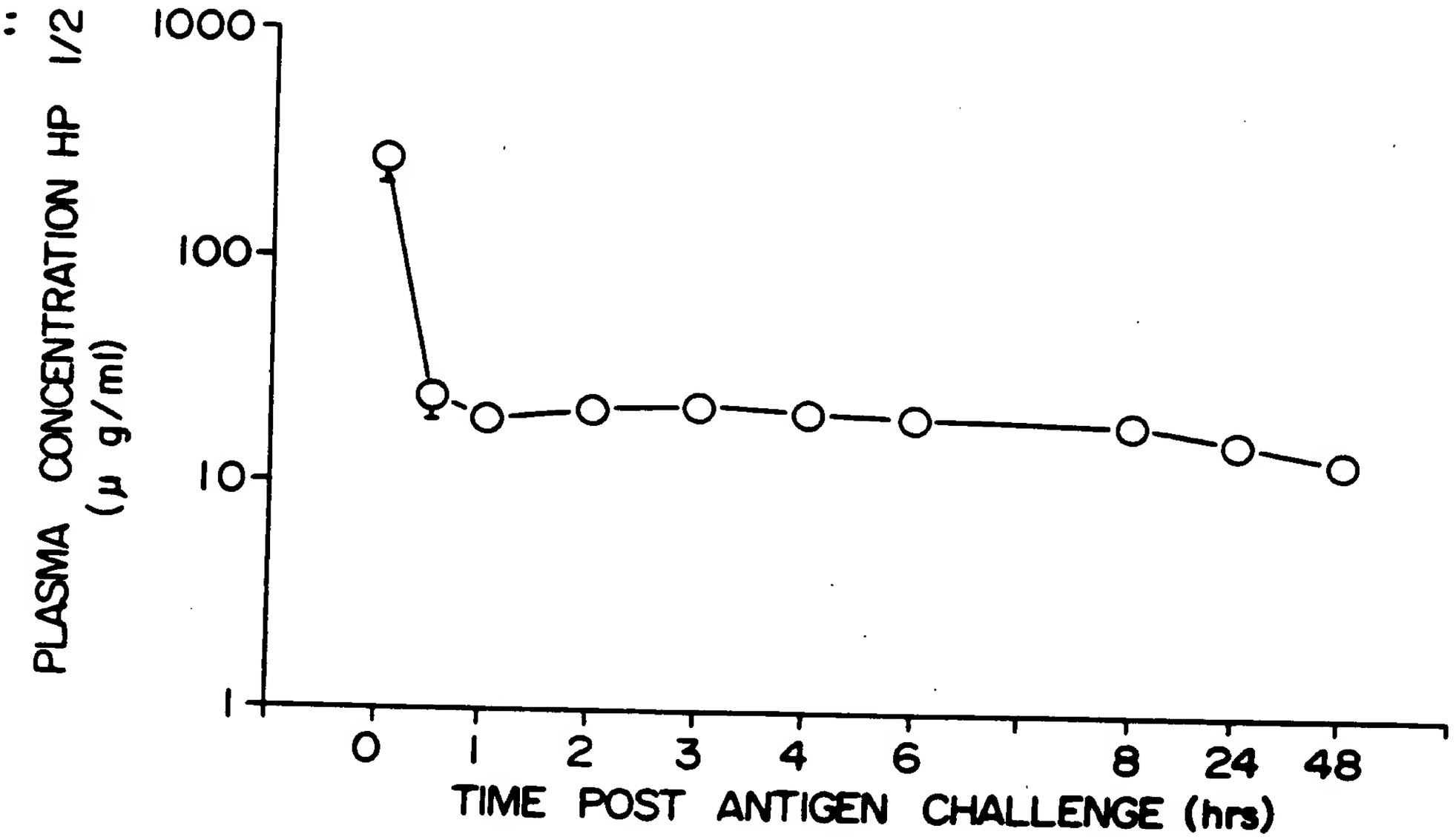

PLASMA CONCENTRATION HP 1/2 (μ g/ml)
1000
100
10
1
0
1
2
3
4
6
8
24
48
TIME POST ANTIGEN CHALLENGE (hrs)

FIG. 2

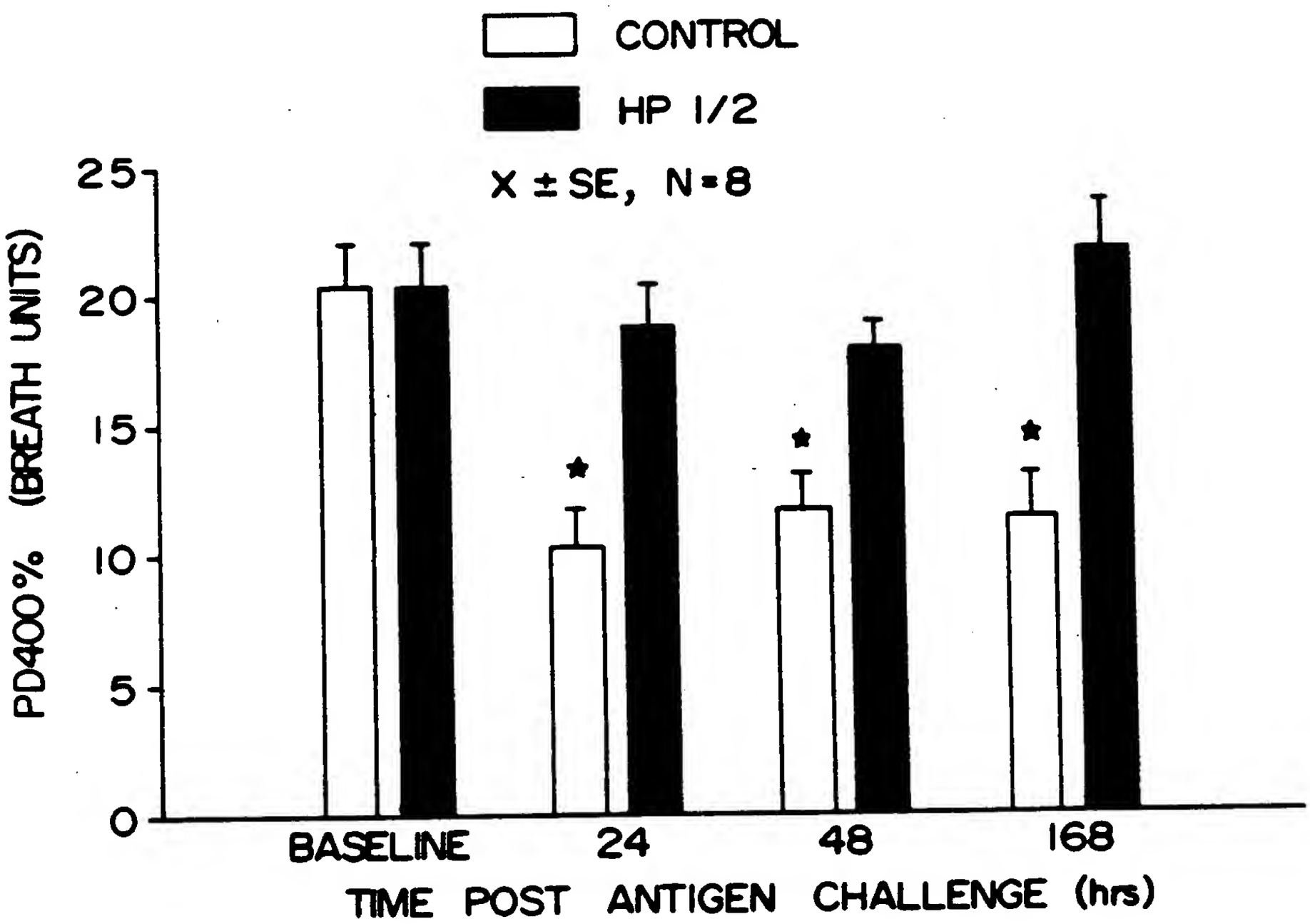

CONTROL
HP 1/2
X ± SE, N=8
PD400% (BREATH UNITS)
25
20
15
10
5
0
BASELINE
24
48
168
TIME POST ANTIGEN CHALLENGE (hrs)

FIG. 3

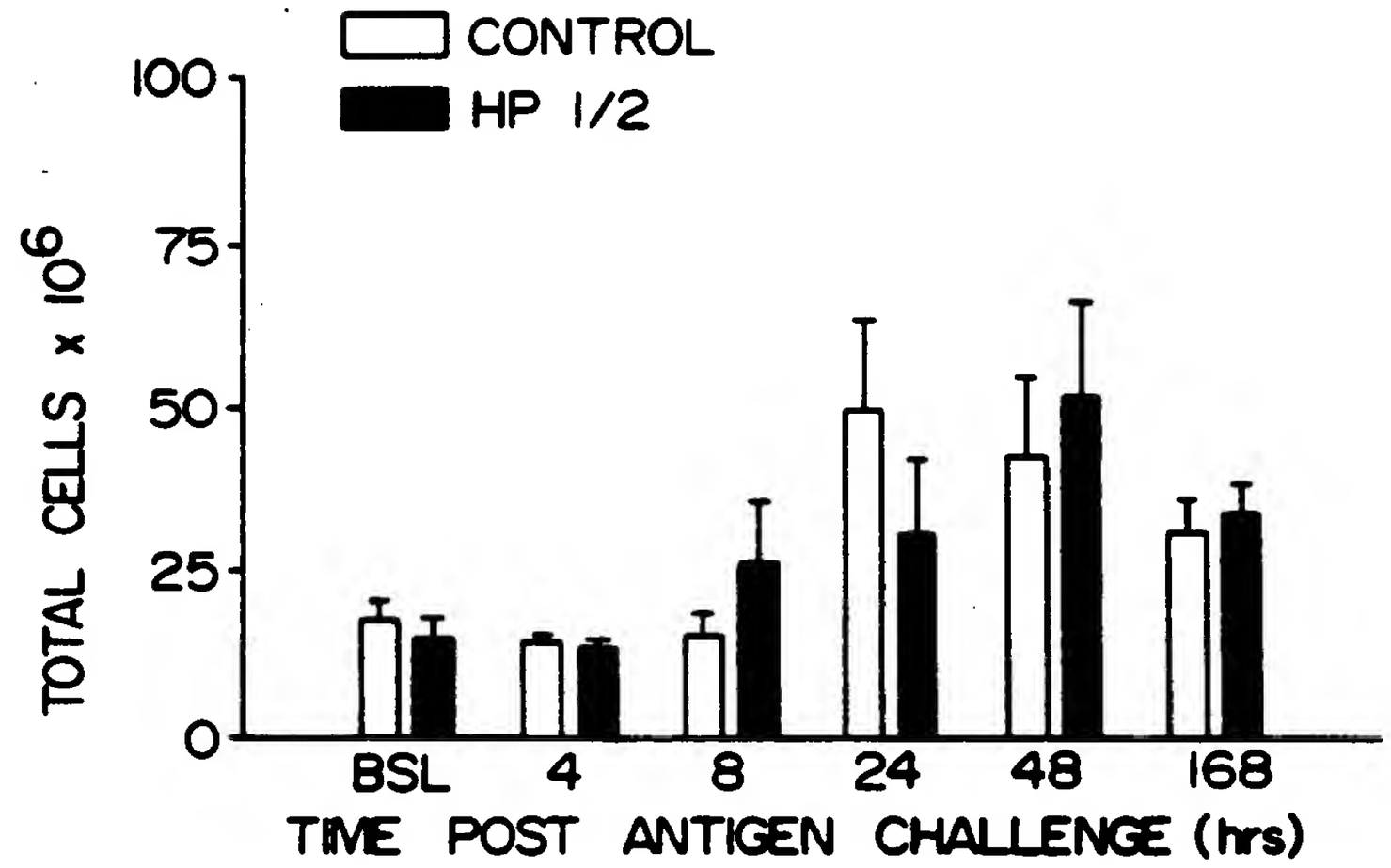

CONTROL
HP 1/2
TOTAL CELLS x 10^6
100
75
50
25
0
BSL
4
8
24
48
168
TIME POST ANTIGEN CHALLENGE (hrs)

FIG. 4A

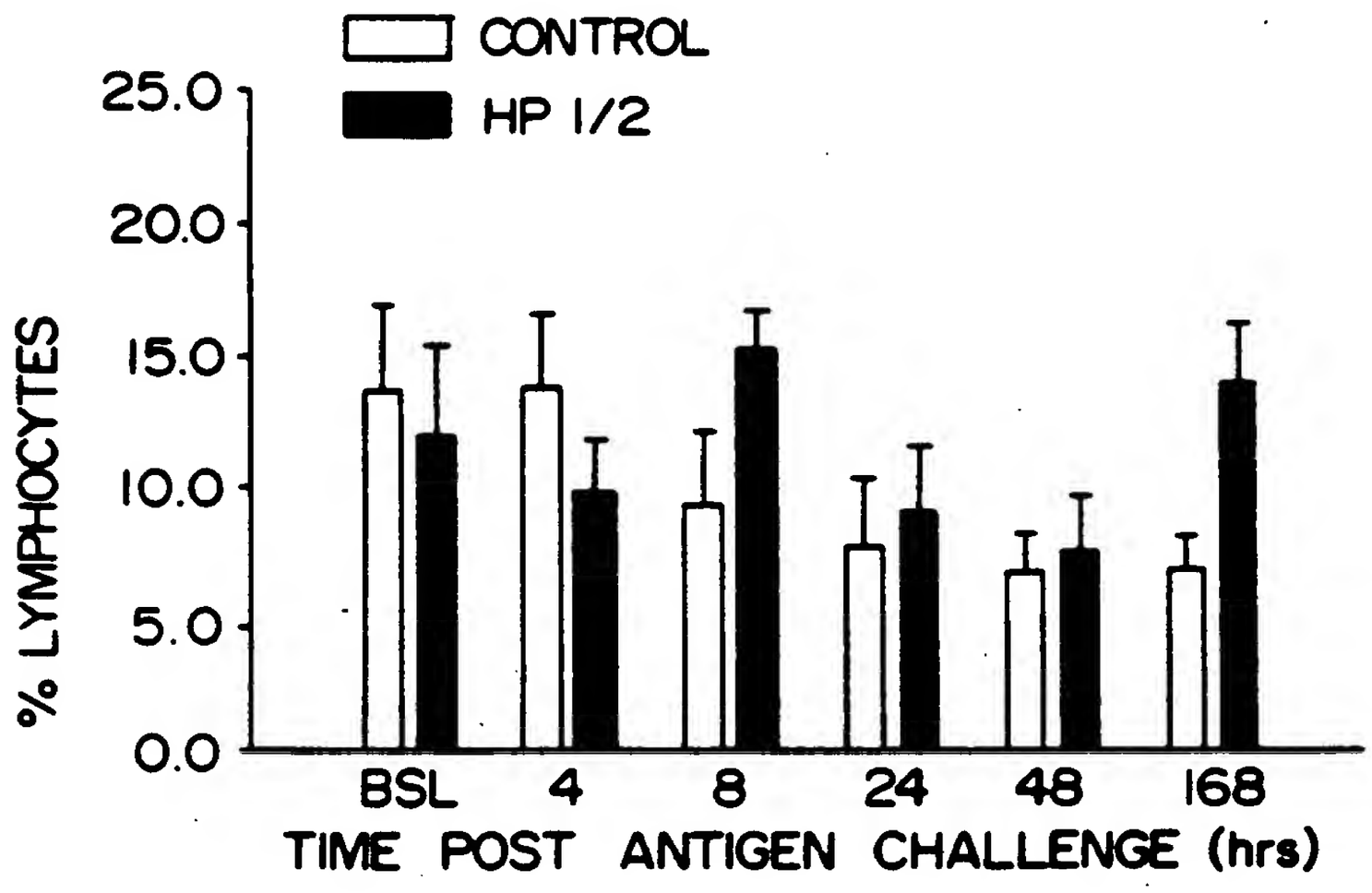

CONTROL
HP 1/2
% LYMPHOCYTES
25.0
20.0
15.0
10.0
5.0
0.0
BSL
4
8
24
48
168
TIME POST ANTIGEN CHALLENGE (hrs)

FIG. 4B

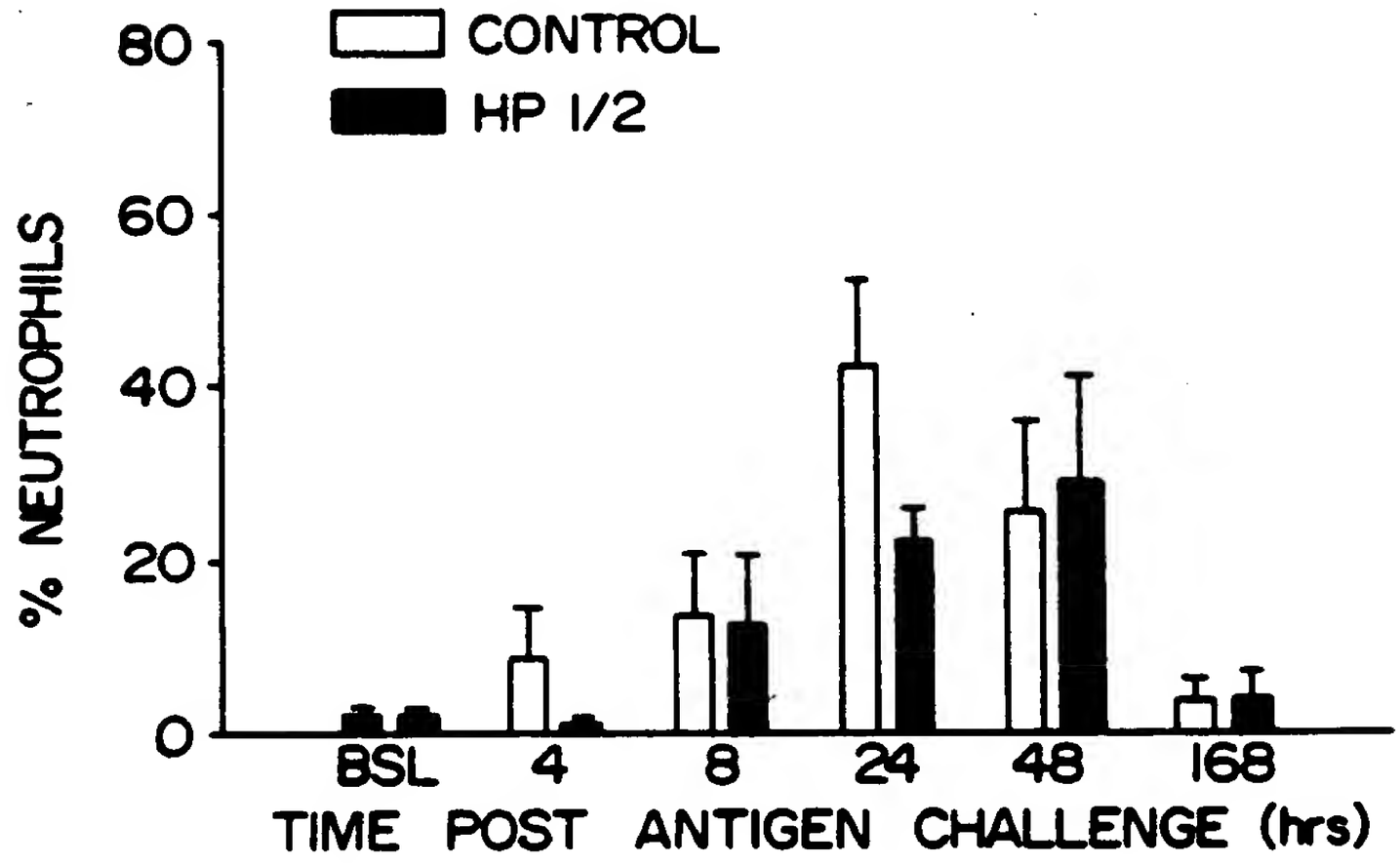

CONTROL
HP 1/2
% NEUTROPHILS
80
60
40
20
0
BSL
4
8
24
48
168
TIME POST ANTIGEN CHALLENGE (hrs)

FIG. 4C

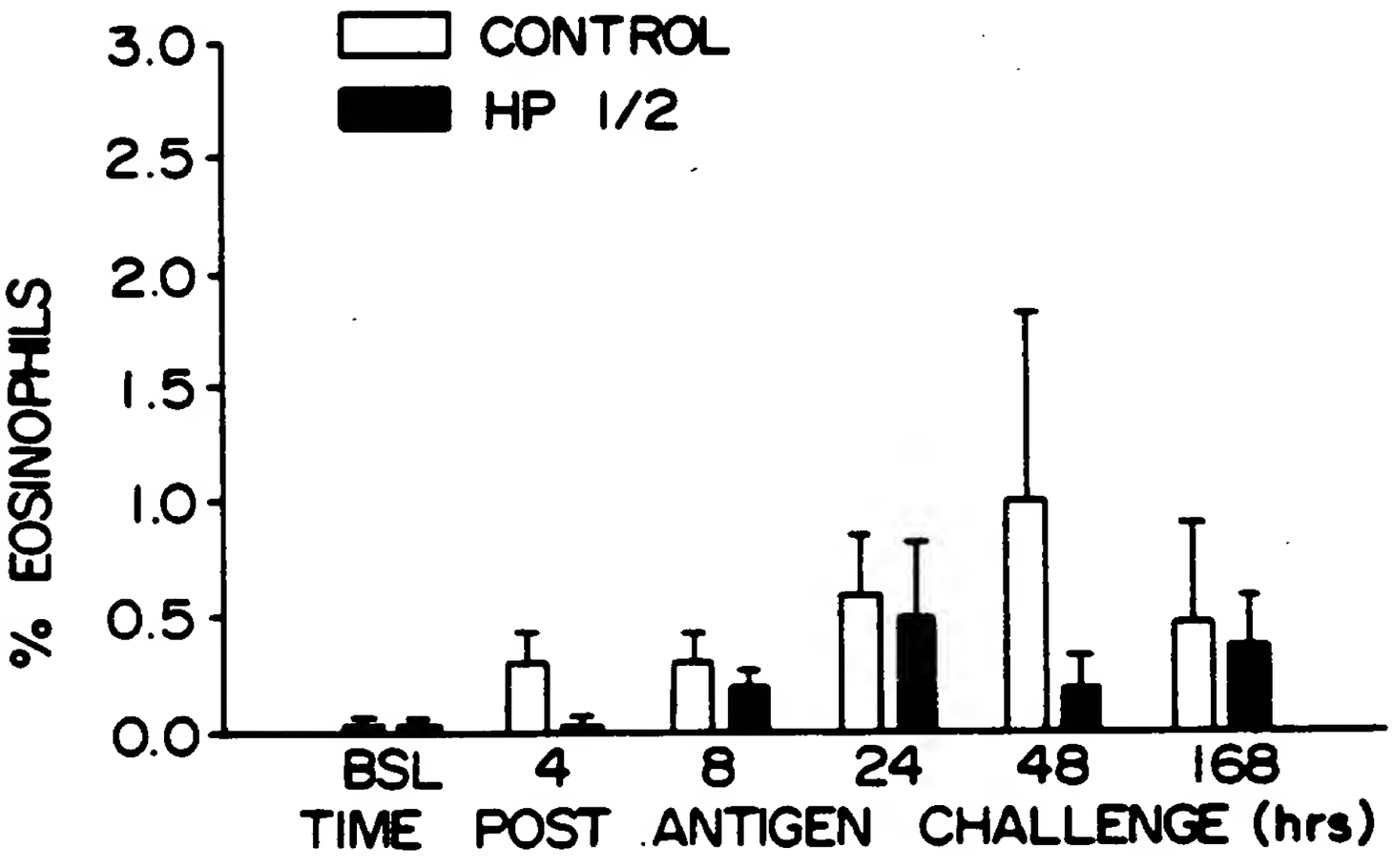
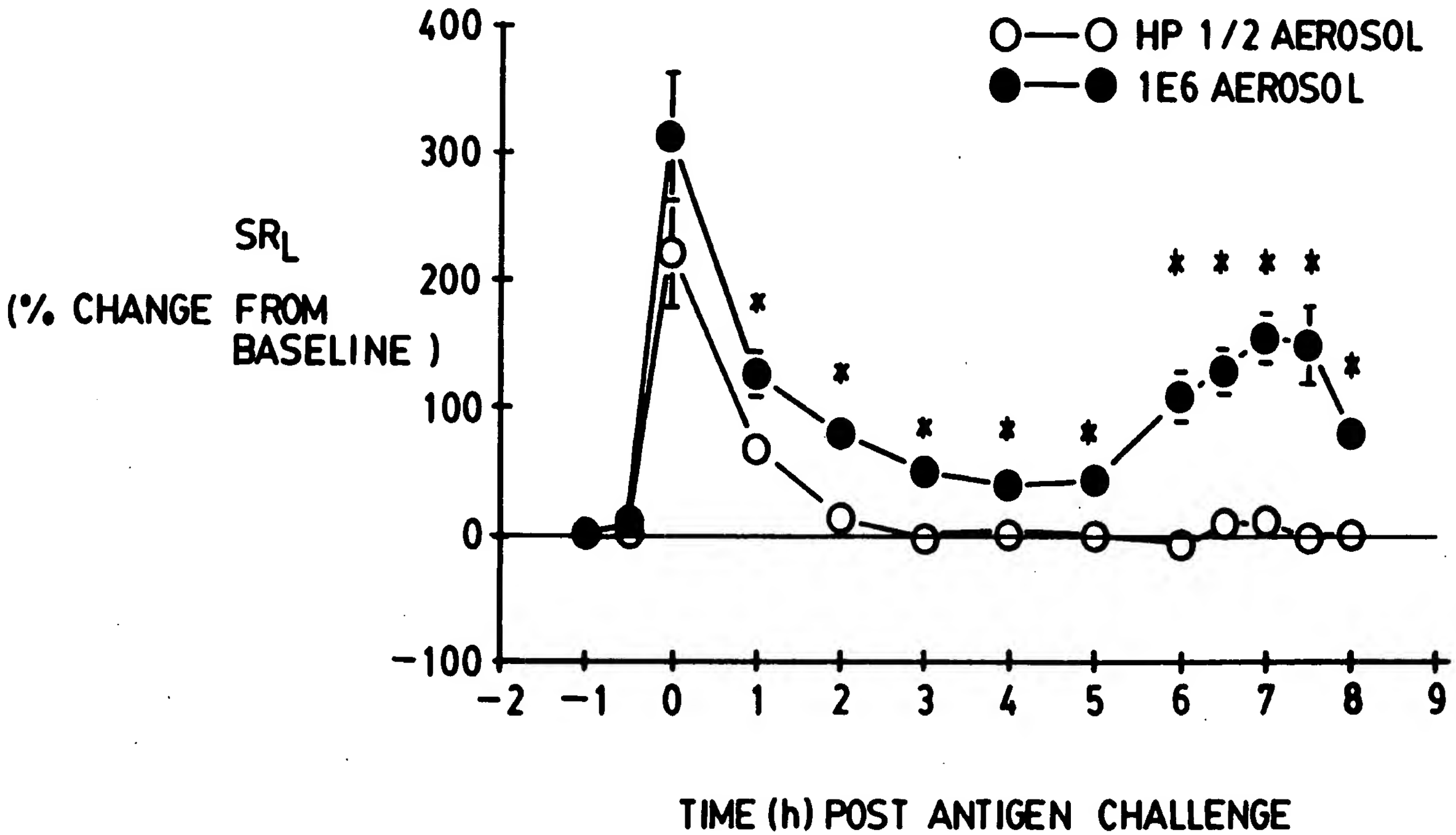

CONTROL
HP 1/2
% EOSINOPHILS
3.0
2.5
2.0
1.5
1.0
0.5
0.0
BSL
4
8
24
48
168
TIME POST ANTIGEN CHALLENGE (hrs)

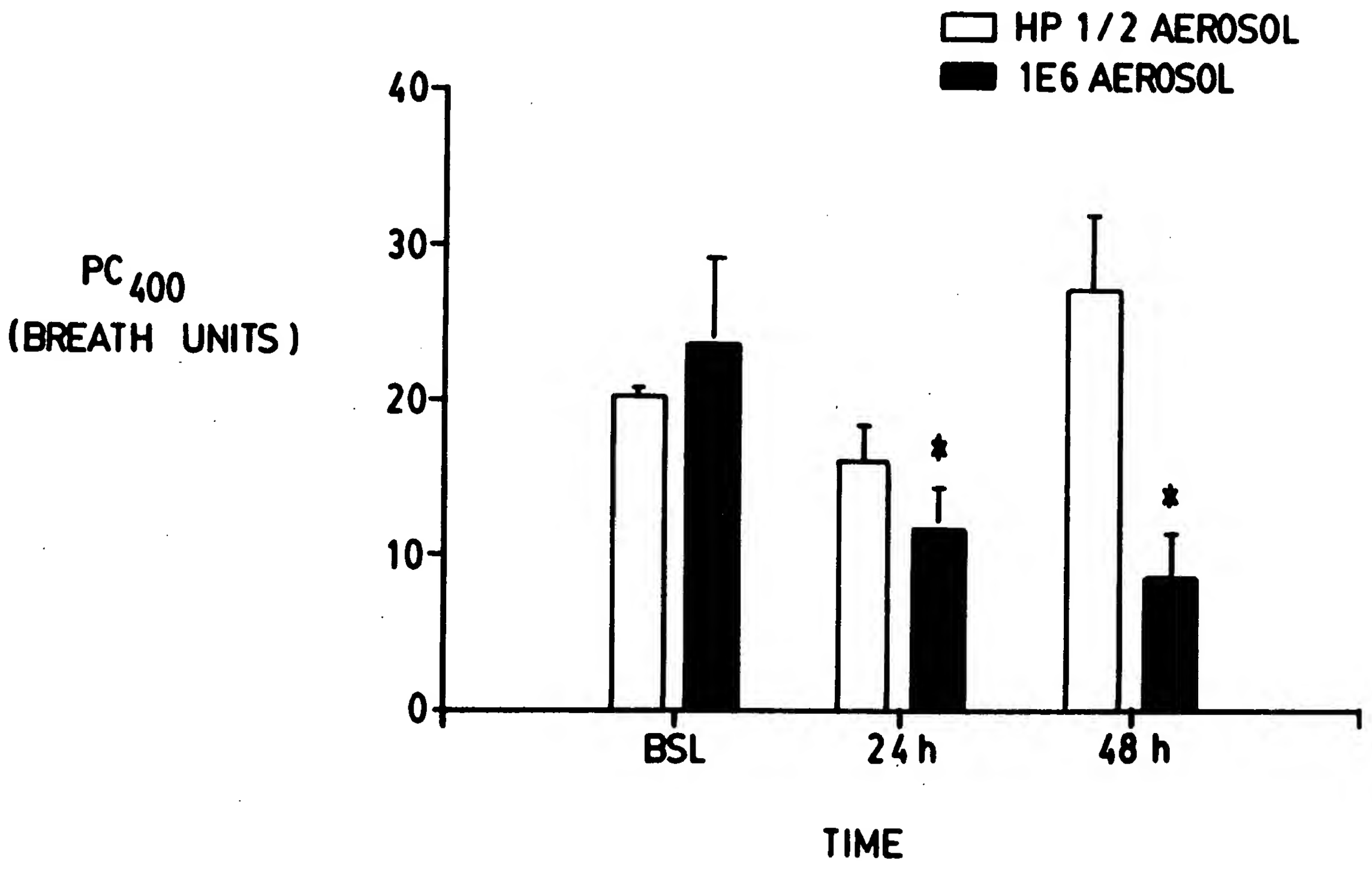
FIG. 4D

FIG. 5



RCT/US 93/00030
26 FEB 1993

FIG. 6



PCT/US 93/00030
RO/US 26 FEB 1993

58 Rec'd PCT/PTO 12 JUL 1994

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
92,307-A; DO(02 CIP PCT)		
International application No.	**International filing date** *(day/month/year)*	**Priority Date** *(day/month/year)*
PCT/US 93/00030	12 January 1993	13 January 1992
Applicant		
BIOGEN, INC. et al.		

This international search report has been prepared by this International Search Authority ans is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] Certain claims were found unsearchable (see Box I).

2. [ ] Unity of invention is lacking (see Box II).

3. [X] The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [X] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority

4. With regard to the title,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is:

   Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/00030

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A2, 0 330 506 (DANA-FABER CANCER INSTITUTE) 30 August 1989 (30.08.89), claims. --	20
P,X	WO, A1, 92/00 751 (NOVO NORDISK A/S) 23 January 1992 (23.01.92), claims 1,7,10-15,20,24,25,27. --	20
A	CHEMICAL ABSTRACTS, vol. 115, no. 23, issued 1991, December 09 (Columbus, Ohio, U.S.A.), S. PARMENTIER et al. "Role of glycoprotein IIa (beta1 subunit of very late activation antigens) in platelet functions", page 539, the abstract-no. 252 791h, Blood 1991, 78(8), 2021-6. --	20

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11 May 1993	01-06-1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SCHNASS e.h.

INTERNATIONAL SEARCH REPORT

Internati application No.
PCT/US 93/00030

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:

Article 17(2)b) & Rule 39.1(iv) PCT

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 114, no. 9, issued 1991, March 04 (Columbus, Ohio, U.S.A.), R. ONISH et al. "A monoclonal antibody, 2H7, which defines a new very late activation antigen, inhibits IL-2-mediated cell proliferation", page 545, the abstract-no. 79 730s, Nippon Ketsueki Gakkai Zasshi 1990, 53(6), 951-63. --	20
A	CHEMICAL ABSTRACTS, vol. 106, no. 5, issued 1987, February 02 (Columbus, Ohio, U.S.A.), H.G. BLUESTEIN et al. "Immunopathogenesis of the neuropsychiatric manifestations of systematic lupus erythematosus", page 395, the abstract-no. 31 234r, Springer Semin. Immunopathol. 1986, 9(2-3), 237-49. ----	20

NOTES TO FORM PCT/ISA/220

These notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty and of the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbufication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

The claims only.

The description and the drawings may only be amended during international preliminary examination under Chapter II.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments wil be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confounded with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
92,307-A; DO(02 CIP PCT)		
International application No.	**International filing date** *(day/month/year)*	**(Earliest) Priority Date** *(day/month/year)*
PCT/US 93/00030	12 January 1993	13 January 1992
Applicant		
BIOGEN, INC. et al.		

This international search report has been prepared by this International Search Authority ans is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] Certain claims were found unsearchable (see Box I).

2. [ ] Unity of invention is lacking (see Box II).

3. [X] The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [X] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority

4. With regard to the title,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is:

   Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/00030

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A2, 0 330 506 (DANA-FABER CANCER INSTITUTE) 30 August 1989 (30.08.89), claims. --	20
P,X	WO, A1, 92/00 751 (NOVO NORDISK A/S) 23 January 1992 (23.01.92), claims 1,7,10-15,20,24,25,27. --	20
A	CHEMICAL ABSTRACTS, vol. 115, no. 23, issued 1991, December 09 (Columbus, Ohio, U.S.A.), S. PARMENTIER et al. "Role of glycoprotein IIa (beta1 subunit of very late activation antigens) in platelet functions", page 539, the abstract-no. 252 791h, Blood 1991, 78(8), 2021-6. --	20

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11 May 1993	01 -06- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SCHNASS e.h.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 114, no. 9, issued 1991, March 04 (Columbus, Ohio, U.S.A.), R. ONISH et al. "A monoclonal antibody, 2H7, which defines a new very late activation antigen, inhibits IL-2-mediated cell proliferation", page 545, the abstract-no. 79 730s, Nippon Ketsueki Gakkai Zasshi 1990, 53(6), 951-63. --	20
A	CHEMICAL ABSTRACTS, vol. 106, no. 5, issued 1987, February 02 (Columbus, Ohio, U.S.A.), H.G. BLUESTEIN et al. "Immunopathogenesis of the neuropsychiatric manifestations of systematic lupus erythematosus", page 395, the abstract-no. 31 234r, Springer Semin. Immunopathol. 1986, 9(2-3), 237-49. ----	20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 93/00030

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:

Article 17(2)b) & Rule 39.1(iv) PCT

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif á la demande de brevet international n°

PCT/US 93/00030 SAE 68912

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les reseignements fournis sont donnés á titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 330506	30-08-89	EP A3 330506 JP A2 2003700	20-06-90 09-01-90
WO A1 9200751	23-01-92	AU A1 82055/91 DK A0 1628/90	04-02-92 06-07-90

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lobb, Roy R.

(ii) TITLE OF INVENTION: Treatment for Asthma

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Allegretti & Witcoff, Ltd.
(B) STREET: 10 South Wacker Drive, Suite 3000
(C) CITY: Chicago
(D) STATE: IL
(E) COUNTRY: US
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT
(B) FILING DATE: 12 January 1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: McNicholas, Janet M.
(B) REGISTRATION NUMBER: 32,918
(C) REFERENCE/DOCKET NUMBER: 92,307-A; D002 CIP PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312-715-1000
(B) TELEFAX: 312-715-1234

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG159 insert: HP1/2 heavy chain variable region; amino acid 1 is Glu (E) but Gln (Q) may be substituted"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GTC AAA CTG CAG CAG TCT GGG GCA GAG CTT GTG AAG CCA GGG GCC TCA        48
Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser
 2               6                  11                  16

GTC AAG TTG TCC TGC ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT        96
Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr
            21                  26                  31

ATG CAC TGG GTG AAG CAG AGG CCT GAA CAG GGC CTG GAG TGG ATT GGA       144
Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
        36                  41                  46

AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC CCG AAG TTC CAG       192
Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln
    51                  56                  61

GTC AAG GCC ACT ATT ACA GCG GAC ACG TCC TCC AAC ACA GCC TGG CTG       240
Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
66                  71                  76                  81

CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT GCC GTC TAC TAC TGT GCA       288
Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                86                  91                  96

GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC TTC TGG GGC CAA       336
Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln
            101                 106                 111

GGG ACC ACG GTC ACC GTC TCC TCA                                      360
Gly Thr Thr Val Thr Val Ser Ser
        116                 121
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser
  2                   6                  11                  16

Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr
              21                  26                  31

Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
          36                  41                  46

Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln
      51                  56                  61

Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
  66                  71                  76                  81

Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                  86                  91                  96

Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln
              101                 106                 111

Gly Thr Thr Val Thr Val Ser Ser
          116                 121
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 318 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..318
(D) OTHER INFORMATION: /product= "HP1/2 light chain variable region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG172 insert: HP1/2 light chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTT TCA GCA GGA          48
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
 1               5                  10                  15

GAC AGG GTT ACC ATA ACC TGC AAG GCC AGT CAG AGT GTG ACT AAT GAT          96
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
            20                  25                  30

GTA GCT TGG TAC CAA CAG AAG CCA GGG CAG TCT CCT AAA CTG CTG ATA         144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45

TAT TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT CGC TTC ACT GGC         192
Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60

AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG GCT         240
Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
65                  70                  75                  80

GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCG TAC         288
Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr
                85                  90                  95

ACG TTC GGA GGG GGG ACC AAG CTG GAG ATC                                 318
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45
```

```
Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
     50                  55                  60

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
 65                  70                  75                  80

Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr
                 85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
            100                 105
```

from 0.05 to 5.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

18. The method of Claim 17, wherein the composition is administered at a dosage so as to provide 1.0-2.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

19. The method according to Claim 12, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the mammal of at least 10 μg/ml over a period of 7 days.

20. A pharmaceutical composition effective to attenuate late phase response or significantly reduce airway hypersensitivity in an asthmatic mammal consisting essentially of a monoclonal antibody recognizing VLA-4 in a pharmaceutically acceptable carrier.

PATENT COOPERATION TREATY

PCT Rec'd PCT/PTO 12 JUL 1994

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
92,307-A		
International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/US 93/00030	12/01/1993	13/01/1992
International Patent Classification (IPC) or national classification and IPC		
A61K39/395		
Applicant		
BIOGEN INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings amended during international preliminary examination and/or containing rectifications made before this Authority.

   These annexes consists of a total of 7 sheets.

3. This report contains indications and corresponding pages relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [X] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/08/1993	13.04.94
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+ 49-89) 2399-0, Tx: 523656 epmu d Fax: (+ 49-89) 2399-4465	M. Herrero

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

I. Basis of the report

1. This report has been drawn up on the basis of:

[ ] the international application as originally filed.

[x] the description, pages 1-5, 7-25_____, as originally filed,
pages _____, filed with the demand,
pages 26-30_____, filed with the letter of 21.04.93,
pages 6A_____, filed with the letter of 11.01.94,

[x] the claims, No. 1-17(part)_____, as originally filed,
No. _____, as amended under Article 19,
No. _____, filed with the demand,
No. 17(part)-21_____, filed with the letter of 11.01.94,
No. _____, filed with the letter of _____,

[x] the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig 1/6-6/6 (RO/US)_____, filed with the letter of 26.02.93
sheets/fig _____, filed with the letter of _____

2. The amendments have resulted in the cancellation of: pages: _____
sheets of drawings/figures No.: _____.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed:

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

III. Non-establishment of opinion with regard to ~~novelty, inventive step and~~ industrial applicability

The question whether the claimed invention appears ~~to be novel, to involve an inventive step (to be non-obvious), or~~ to be industrially applicable ~~have~~ has not been and will not be examined in respect of:

[ ] the entire international application,

[x] claims Nos. 1-20

because:

[x] the said international application, or the said claims Nos. 1-20 relate to the following subject matter which does not require an international preliminary examination (specify):

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods (PCT Rule 67.1(iv)). See also Section V, Citations and Explanations.

[ ] the description, claims or drawings (indicate particular elements below) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (specify):

[ ] the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

[ ] no international search report has been established for said claims Nos. _____.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-21	YES
	Claims	NO
Inventive Step (IS)	Claims 1-21	YES
	Claims	NO
Industrial Applicability (IA)	Claims 21	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

1. The subject-matter at present claimed meets the criteria set forth in Article 33(2)-33(3) PCT since the available prior art does not teach or suggest the claimed invention.

The application discloses a method for treating asthma in a mammal which method relies on the blockage of the VCAM1/VLA-4 adhesion pathway of circulating leukocytes and employs substances which specifically interfere with VLA-4-mediated binding. Molecules contemplated as capable of inhibiting or blocking said VLA-4 mediated binding are in particular anti-VLA-4 antibodies and VLA-4 binding fragments thereof capable of binding to the α_4 subunit of VLA-4, and certain polypeptides, e.g. soluble VCAM-1 or fragments thereof which compete for the VLA-4 binding site, and oligosaccharides which mimic the binding domain of a VLA-4 ligand.

2. For the assessment of the present Claims 1-20 on the question whether they are industrially applicable, no

unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. It is clearly pointed out in the description (see for instance page 12, lines 15-17) that for the purposes of the present invention, antibodies capable of binding to the α_4 subunit of VLA-4 must be employed. Since independent Claim 1 does not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6(3)(b) PCT that any independent claim must contain all the technical features essential to the invention.

2. The terms "a polypeptide" and "a small molecule" as presently used in the independent Claim 12 are vague and indefinite and, as such, render the scope of the claim unclear; accordingly, the claim does not satisfy the requirement of Article 6 PCT. In the light of the description it could be suggested to clarify said vague terms using the corresponding expressions "a polypeptide which competes for the VLA-4 binding site" (page 13, line 26) and "a small molecule that mimic the binding domain of a VLA-4 ligand" (page 13, lines 29-30).

Best Available Copy

58 Rec'd PCT/PTO 12 JUL 1994

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Mrs J.M. McNicholas
ALLEGRETTI & WITCOFF, LTD.
10 South Wacker Drive
CHICAGO, ILLINOIS 60606
ETATS-UNIS D'AMERIQUE

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	13.10.93
Applicant's or agent's file reference: 92,307-A	REPLY DUE within 3 months/days from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 93/00030	12/01/1993	13/01/1992

International Patent Classification (IPC) or both national classification and IPC
A61K39/395

Applicant
BIOGEN INC. et al.

Written Opinion, if any, due: January 13, 1994

1. This written opinion is the FIRST (first, etc.) drawn up by this International Preliminary Examining Authority.

2. This report contains indications and corresponding pages relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [X] Certain observations on the international application

3. The applicant is **hereby invited to reply** to this opinion.

When? **See the time limit indicated above.** The applicant may, before the expiration of that time limit, request this Authority **to grant an extension.**

How? **By submitting a written reply,** accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4*bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 13/05/1994

Name and mailing address of the IPEA/	Authorized officer / Examiner
European Patent Office D-80298 Munich Tel. (+ 49-89) 2399-0, Tx: 523656 epmu d Fax: (+ 49-89) 2399-4465	M. Herrero
	Formalities officer (incl. extension of time limits)

WRITTEN OPINION

I. Basis of the opinion

1. This opinion has been drawn up on the basis of:

[ ] the international application as originally filed.

[x] the description, pages 1-25_____, as originally filed,
pages _____, filed with the demand,
pages 26-30_____, filed with the letter of 21.04.93,

[x] the claims, No. 1-20_____, as originally filed,
No. _____, as amended under Article 19,
No. _____, filed with the demand,
No. _____, filed with the letter of _____,

[x] the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig 1/6-6/6_____, filed with the letter of 26.02.93,

2. The amendments have resulted in the cancellation of: pages: _____
sheets of drawings/figures No.: _____.

3. [ ] This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed:

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to ~~novelty, inventive step and~~ industrial applicability

The question whether the claimed invention appears ~~to be novel, to involve an inventive step (to be non-obvious), or~~ to be industrially applicable ~~have~~ has not been and will not be examined in respect of:

[ ] the entire international application,

[x] claims Nos. 1-19

because:

[x] the said international application, or the said claims Nos. 1-19 relate to the following subject matter which does not require an international preliminary examination (specify):

Methods for treatment of the human of animal body by surgery or therapy, as well as diagnostic methods (PCT Rule 67.1 (iv)). See also Section V, Citations and Explanations, item 2.4.

[ ] the description, claims or drawings (indicate particular elements below) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (specify):

[ ] the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

[ ] no international search report has been established for said claims Nos. _____.

WRITTEN OPINION

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims _____
	Claims _____
Inventive Step (IS)	Claims 1-4, 6-12, 14, 15, 17-20
	Claims _____
Industrial Applicability (IA)	Claims 1-19? (see item 2.4 below)
	Claims _____

2. CITATIONS AND EXPLANATIONS

2.1 The following documents are mentioned for the first time in this written opinion; the numbering will be adhered to in the rest of the procedure:

D1 = Weller, P.F. et al (1991) Proc. Natl. Acad. Sci. USA 88:7430-7433

D2 = Wegner, C.D. et al (1990) Science 247:456-459

(Documents D1 and D2 have been cited in the description and respectively appear in the list of cited publications as references 10 and 28. A copy of both documents is enclosed with the communication).

2.2 As stated in the description, it is known from the prior art that one of the characteristics of the allergen-induced asthma response is the recruitment of inflammatory leukocytes to inflamed lung tissue. More in particular, the late phase response in allergen-induced asthma and persistent hyperresponsiveness have been associated with the recruitment of eosinophils (see page

3, second paragraph).

It had been previously established that, contrary to neutrophils, eosinophils express the VLA-4 (very late antigen-4), i.e. the receptor for VCAM-1 and that within the inflammatory response, eosinophils participate in three distinct cell adhesion pathways to vascular endothelium, binding to cells expressing intercellular adhesion molecule-1 (ICAM-1), endothelial cell adhesion molecule-1 (ELAM-1), and vascular cell adhesion molecule-1 (VCAM-1) (see in the description page 4, lines 24-27 and 14-19).

From document D1 it was known that eosinophil adherence to VCAM-1 might provide a mechanism contributing to the selective recruitment of eosinophils into tissue sites of inflammation. Referring to the accumulation of eosinophils without neutrophils in specific inflammatory sites D1 indicates in page 7432, right column, last paragraph over page 7433, that the expression by human eosinophils of VLA-4 and its role in mediating their adhesion to VCAM-1 could contribute to the concomitant accumulation of eosinophils noted in some immune reactions, such as in airway tissues of those with asthma (emphasis added).

Furthermore, at page 7433, left column, last paragraph D1 makes reference to the fact previously demonstrated in document D2, that the administration of MoAbs against ICAM-1 to primates, prior to inhalational antigen challenges, diminished both eosinophil infiltration and bronchial hyperreactivity induced in a primate model of asthma. At that point, and in view of the results shown in Fig. 3 of page 7432 (where the MoAb HP2/1 is employed), D1 suggests that suitable MoAb combinations -e.g. against CD18 and VLA-4- could be highly effective in inhibiting eosinophil recruitment "in vivo".

Having regard to the foregoing it seems that the skilled person facing the problem of finding a blocking activity for the inflammatory response in asthma would have followed the indications of D1 and, focusing the attention on a eosinophil adhesion pathway different of the CD11a-CD18/ICAM-1 tested in D2, would have also made use of known MoAbs to test on the VLA-4/VCAM-1 pathway. The suitability of some of said MoAbs to solve the posed problem would have then been established by means of using already available MoAbs and standard techniques involving only trial and error.

It follows that the subject-matter of present independent Claims 1, 12 and 20 referring to the use of a generic MoAb recognizing VLA-4 for the treatment of asthma, does not involve an inventive step contrary to Art. 33(3) PCT. The same objection applies to the dependent Claims 2-4, 6-11, 14, 15 and 17-19.

2.3 On the other hand, the subject-matter claimed involving the administration of the specific MoAb HP1/2 (present Claim 5, Claim 13 in part, and Claim 16), which in its turn corresponds to the only MoAb recognizing VLA-4 exemplified/characterized in the application, is considered to comply with the requirements of Art. 33(3) PCT, as the use of said MoAb HP1/2 represents a non-arbitrary selection for which an advantageous effect, not expected by the skilled person, has been demonstrated (see in this respect page 20, lines 9-15).

2.4 For the assessment of the present Claims 1-19 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to

the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The apparent common concept that links the subject-matter according to the present application is regarded as the possibility of using a MoAb recognizing VLA-4 in the treatment of asthma.

It follows that the claimed embodiments corresponding to the use for the same final purpose of "a polypeptide or a small molecule capable of binding to the alpha subunit of VLA-4" (Claim 12) or "soluble VCAM-1 polypeptides; or small molecules that bind to the VCAM-1-binding domain of VLA-4" (Claim 13) or "polypeptide or small molecule" (Claims 17 and 18) are regarded as subject-matter non-unitary with the rest of the subject-matter entitled to be claimed (Rule 13 PCT).